

PCT

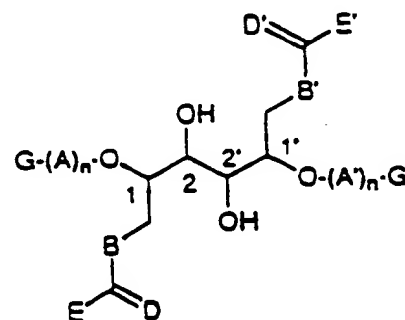
WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁵: C07C 271/22, A61K 31/27, C07D 213/80, 285/16, C07C 327/28, C07D 295/21, C07C 333/20, A61K 31/44, 31/41, 31/265	A1	(11) International Publication Number: WO 94/13629 (43) International Publication Date: 23 June 1994 (23.06.94)
(21) International Application Number: PCT/US93/12059 (22) International Filing Date: 9 December 1993 (09.12.93) (30) Priority Data: 07/989,239 11 December 1992 (11.12.92) US (60) Parent Application or Grant (63) Related by Continuation US 07/989,239 (CIP) Filed on 11 December 1992 (11.12.92) (71) Applicant (for all designated States except US): VERTEX PHARMACEUTICALS INCORPORATED [US/US]; 40 Allston Street, Cambridge, MA 02139-4211 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): BOGER, Joshua, S. [US/US]; 243 Old Pickard Road, Concord, MA 01742 (US). MEYERS, Harold, V. [US/US]; 46 Van Ness Road, Bel- mont, MA 02178 (US). MULLICAN, Michael, D. [US/US]; 110 Parker Road, Needham, MA 02194 (US).		(74) Agents: PIERRI, Margaret, A. et al.; Fish & Neave, 1251 Avenue of the Americas, New York, NY 10020 (US). (81) Designated States: AT, AU, BB, BG, BR, BY, CA, CH, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, KZ, LK, LU, LV, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SK, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published With international search report.

(54) Title: MANNITOL DERIVATIVES AND THEIR USE AS INHIBITORS OF ASPARTYL PROTEASE**(57) Abstract**

The present invention relates to a class of compounds which are aspartyl protease inhibitors. In one embodiment, this invention relates to a novel class of mannitol-derived HIV aspartyl protease inhibitors characterized by specific structural and physicochemical features represented by formula (I), each A and A' is independently selected from the group consisting of a naturally occurring alpha-amino acid and an unnatural alpha-amino acid (e.g., wherein each n is independently selected from the group consisting of 0, 1 and 2; wherein each B and B' is independently selected from the group consisting of oxygen and sulfur; wherein each D and D' is independently selected from the group consisting of H₂, oxygen and sulfur, wherein each E and E' is independently selected from the group consisting of Ar and N (R₁₁R₁₂). The compounds and pharmaceutical compositions of this invention are particularly well suited for inhibiting the activity of HIV aspartyl protease. Accordingly, they may be advantageously used as anti-viral agents against HIV viruses, including the HIV-1 and HIV-2 viruses.



(I)

Best Available Copy

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

- 1 -

Mannitol derivatives and their use as inhibitors of aspartyl protease

TECHNICAL FIELD OF THE INVENTION

The present invention relates to a novel class of compounds which are aspartyl protease inhibitors. In one embodiment, this invention relates to a novel class of mannitol-derived HIV aspartyl protease inhibitors characterized by specific structural and physicochemical features. This invention also relates to pharmaceutical compositions comprising these compounds. The compounds and pharmaceutical compositions of this invention are particularly well suited for inhibiting the activity of HIV aspartyl protease. Accordingly, they may be advantageously used as anti-viral agents against HIV viruses, including the HIV-1 and HIV-2 viruses.

BACKGROUND OF THE INVENTION

The human immunodeficiency virus ("HIV") retrovirus is the causative agent for acquired immunodeficiency syndrome ("AIDS") -- a disease characterized by the destruction of the immune system, particularly of CD4⁺ T-cells (J. of NIH Res., 3, pp. 23-25 (1991)), with attendant susceptibility to opportunistic infections; its precursor AIDS-related complex ("ARC") -- a syndrome characterized by symptoms such as persistent generalized lymphadenopathy, fever,

- 2 -

weight loss; and its precursor -- asymptomatic HIV infection.

As in the case of several other retroviruses, HIV encodes the production of a protease which carries out post-translational cleavage of precursor polypeptides in a process necessary for the formation of infectious virions (S. Crawford et al., "A Deletion Mutation in the 5' Part of the pol Gene of Moloney Murine Leukemia Virus Blocks Proteolytic Processing of the gag and pol Polyproteins", J. Virol., 53, p. 899 (1985)). These gene products include pol, which encodes the virion RNA-dependent DNA polymerase (reverse transcriptase), an endonuclease, HIV protease, and gag, which encodes the core-proteins of the virion (H. Toh et al., "Close Structural Resemblance Between Putative Polymerase of a Drosophila Transposable Genetic Element 17.6 and pol gene product of Moloney Murine Leukemia Virus", EMBO J., 4, p. 1267 (1985); L.H. Pearl et al., "A Structural Model for the Retroviral Proteases", Nature, pp. 329-351 (1987); M.D. Power et al., "Nucleotide Sequence of SRV-1, a Type D Simian Acquired Immune Deficiency Syndrome Retrovirus", Science, 231, p. 1567 (1986)).

A number of synthetic anti-viral agents have been designed to target various stages in the replication cycle of HIV. These agents include compounds which block viral binding to CD4⁺ T-lymphocytes (for example, soluble CD4), and compounds which interfere with viral replication by inhibiting viral reverse transcriptase (for example, didanosine and zidovudine (AZT)) and inhibit integration of viral DNA into cellular DNA (M.S. Hirsh and R.T. D'Aquila, "Therapy for Human Immunodeficiency Virus Infection", N. Eng. J. Med., 328, p. 1686 (1993)). However, such agents, which are directed primarily to early stages of

- 3 -

viral replication, do not prevent the production of infectious virions in chronically infected cells. Furthermore, administration of some of these agents in effective amounts has led to cell-toxicity and unwanted side effects, such as anemia and bone marrow suppression.

More recently, the focus of anti-viral drug design has been to create compounds which inhibit the formation of infectious virions by interfering with the processing of viral polyprotein precursors. Processing of these precursor proteins requires the action of virus-encoded proteases which are essential for replication (Science, 231, pp. 1580-1584 (1986); Kohl, N.E. et al. "Active HIV Protease is Required for Viral Infectivity" Proc. Natl. Acad. Sci. USA, 85, p. 4686 (1988)). The anti-viral potential of HIV protease inhibition has been demonstrated using peptidal inhibitors. Such peptidal compounds, however, are typically large and complex molecules that tend to exhibit poor bioavailability and are not generally consistent with oral administration. Accordingly, the need still exists for compounds that can effectively inhibit the action of viral proteases, for use as agents for preventing and treating chronic and acute viral infections, such as HIV.

SUMMARY OF THE INVENTION

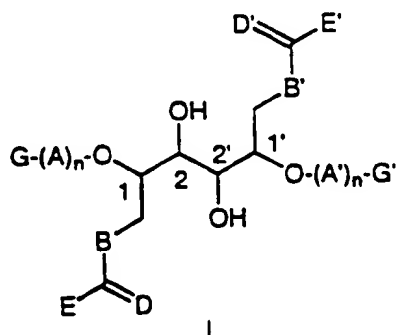
The present invention provides a novel class of compounds, including pharmaceutically acceptable derivatives thereof, that have an affinity for aspartyl proteases, in particular, HIV aspartyl proteases. Based on that affinity, these compounds are useful as inhibitors of such proteases. These compounds can be used alone or in combination with other therapeutic or prophylactic agents, such as anti-virals, antibiotics,

- 4 -

immunomodulators or vaccines, for the treatment or prophylaxis of viral infection.

According to a preferred embodiment, the compounds of this invention are capable of inhibiting HIV viral replication in human CD_4^+ T-cells, by inhibiting the ability of HIV aspartyl proteases to catalyze the hydrolysis of peptide bonds. These novel compounds can thus serve to reduce the production of infectious virions from chronically infected cells, and can inhibit the initial or further infection of host cells. Accordingly, these compounds are useful as therapeutic and prophylactic agents to treat or prevent infection by HIV-1 and related viruses, which may result in asymptomatic HIV-1 infection, AIDS-related complex ("ARC"), acquired immunodeficiency syndrome ("AIDS"), AIDS-related dementia, or similar disease of the immune system.

It is a principal object of this invention to provide a novel class of mannitol derivatives which are aspartyl protease inhibitors, and particularly, HIV aspartyl protease inhibitors. This novel class of mannitol derivatives is represented by formula I:



and pharmaceutically acceptable derivatives thereof, including pharmaceutically acceptable salts thereof, wherein each n is independently selected from the group consisting of 0, 1 and 2;

- 5 -

each A and A' is independently selected from the group consisting of a naturally occurring alpha-amino acid and an unnatural alpha-amino acid (e.g., Ala, Asn, Cys, Gly, Gln, His, Ile, Leu, Met, Phe, Pro, Ser, Thr, Trp, Val or trifluoroalanine), wherein the amino group of each A or A' is bonded to G or G' or to the carboxy group of the adjacent residue A or A', whichever is appropriate, and the carboxy group of A or A' is bonded to the amino group of the adjacent residue A or A' or to the oxygen of the structure, whichever is appropriate;

each G and G' is independently covalently attached to the amino group of the adjacent residue A or A' or to the oxygen of the structure (if the adjacent n = 0), and is selected from the group consisting of:

- 1) trityl;
- 2) hydrogen;
- 3) C₁-C₆ alkyl;
- 4) R₃-CO-,
- 5) phthaloyl, wherein the aromatic ring thereof is optionally substituted with one or more substituents R₄;
- 6) R₅(R₆R₇C)_mCO-, wherein m = 1-3;
- 7) R₅(R₆R₇C)_mW-, wherein m = 1-3 and each W is independently selected from the group consisting of -OCO- and -SO₂-, provided that W is not -SO₂- when W is attached to the oxygen of the structure;
- 8) R₈-W-, provided that W is not -SO₂- when W is attached to the oxygen of the structure;
- 9) R₅(R₆R₇C)_m-P(O)(OR₉)-, wherein m = 1-3; and
- 10) R₈-P(O)(OR₉)-

- 6 -

each R_3 is independently selected from the group consisting of: 1) hydrogen; 2) C_1-C_6 alkyl or C_2-C_6 alkenyl, both of which may be optionally substituted with one or more substituents selected from the group consisting of hydroxyl, chlorine and fluorine; and 3) phenyl or naphthyl, both of which may be optionally substituted with one or more substituents R_4 ;

each R_4 is independently selected from the group consisting of C_1-C_4 alkyl, C_2-C_4 alkenyl, halogen (e.g., F, Cl, Br or I), hydroxyl, nitro, C_1-C_3 alkoxy and $-CO-N(R_{10})(R_{10})$;

each R_5 , R_6 and R_7 is independently selected from the group consisting of hydroxyl; hydrogen; chlorine; fluorine; C_1-C_3 alkoxy; a 5-7 membered heterocycle; C_1-C_3 alkyl; phenyl; and naphthyl; said C_1-C_3 alkyl being optionally substituted with one or more substituents selected from the group consisting of chlorine, fluorine, and hydroxyl; and said phenyl and naphthyl being optionally substituted with one or more substituents R_4 ; or R_5 , R_6 and R_7 of a particular G or G' taken individually, or together in any combination, may be optionally joined to form a monocyclic, bicyclic, or tricyclic ring system, each ring of which is C_3-C_6 cycloalkyl, with the proviso that R_5 , R_6 or R_7 may not be chlorine, fluorine or hydroxyl if it is present on the carbon adjacent to W;

each R_8 is independently selected from the group consisting of phenyl, naphthyl, and a 5-7 membered heterocycle (e.g., pyridyl, furyl or benzisoxazolyl), wherein said phenyl or naphthyl may be substituted with one or more substituents R_4 ;

each R_9 is independently selected from the group consisting of C_1-C_4 alkyl and phenyl;

- 7 -

each R_{10} is independently selected from the group consisting of hydrogen, C_1-C_4 alkyl, and a 5-7 membered heterocycle (e.g., pyridyl, furyl, or benzisoxazolyl);

5 wherein each B and B' is independently selected from the group consisting of oxygen and sulfur;

 wherein each D and D' is independently selected from the group consisting of H_2 , oxygen and
10 sulfur;

 wherein each E and E' is independently selected from the group consisting of Ar and $N(R_{11}R_{12})$;

 wherein each R_{11} and R_{12} is independently selected from the group consisting of C_1-C_6 alkyl and
15 C_2-C_6 alkenyl; and

 wherein each Ar is independently selected from the group consisting of phenyl, phenyl substituted with one or more substituents R_4 and a 5-7 membered heterocycle, optionally substituted with one or more
20 substituents R_4 (e.g., 3-pyridyl, 4-(1,2,3-thiadiazol)-yl, or 4-morpholine).

 It is also an object of this invention to provide pharmaceutical compositions comprising the mannitol derivatives of formula I and methods for their
25 use as inhibitors of aspartyl proteases, including HIV aspartyl proteases.

DETAILED DESCRIPTION OF THE INVENTION

 In order that the invention herein described may be more fully understood, the following detailed
30 description is set forth. In the description, the following abbreviations are used:

<u>Designation</u>	<u>Reagent or Fragment</u>
Et	ethyl
Trityl	triphenylmethyl

- 8 -

	Ala	D- or L-alanine
	Asn	D- or L-asparagine
	Cys	D- or L-cysteine
	Gly	D- or L-glycine
5	Gln	D- or L-glutamine
	His	D- or L-histidine
	Ile	D- or L-isoleucine
	Leu	D- or L-leucine
	Met	D- or L-methionine
10	Phe	D- or L-phenylalanine
	Pro	D- or L-proline
	Ser	D- or L-serine
	Thr	D- or L-threonine
	Trp	D- or L-tryptophan
15	Val	D- or L-valine
	Cbz	benzyloxycarbonyl (carbobenzyloxy)
	EDC	1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride
	TFA	trifluoroacetic acid
20	EtOAc	ethyl acetate
	DMF	dimethylformamide
	AZT	zidovudine
	TIBO	4,5,6,7-tetrahydro-5-methylimidazo[4,5,1-jk][1,4]
25		benzodiazepin-2(1H)-one
	IL-2	interleukin-2
	GM-CSF	granulocyte macrophage colony stimulating factor
	rEPO	recombinant erythropoietin
30	EtOH	ethyl alcohol
	MeOH	methyl alcohol
	THF	tetrahydrofuran
	DMAP	4-dimethylaminopyridine
	Et ₂ O	diethyl ether.

- 9 -

The following terms are employed herein:

Unless expressly stated to the contrary, the term "-P(O)(OR₉)-" as used herein refers to a phosphonate derivative (i.e., both appended groups
5 linked to the P), and not a phosphinate ester.

The term "Ar" refers to either a saturated or an unsaturated moiety.

The term "the oxygen of the structure" refers to the oxygen of a primary hydroxyl group of the
10 mannitol backbone of the compounds of this invention.

The term "heterocycle" refers to a stable 5-7 membered monocycle or 5-7 membered bicyclic heterocycle, which is either saturated or unsaturated, and which may be optionally benzofused if monocyclic.
15 Each heterocycle consists of carbon atoms and from one to four heteroatoms selected from the group consisting of nitrogen, oxygen and sulfur. As used herein, the terms "nitrogen and sulfur heteroatoms" include any oxidized form of nitrogen and sulfur, and the
20 quaternized form of any basic nitrogen. The heterocyclic ring may be attached by any heteroatom or carbon atom of the cycle which results in the creation of a stable structure. Preferred heterocycles defined above include, but are not limited to, benzimidazolyl, imidazolyl, imidazolinoyl, imidazolidinyl, quinolyl, isoquinolyl, indolyl, pyridyl, pyrrolyl, pyrrolinyl, pyrazolyl, pyrazinyl, quinoxolyl, piperidinyl, morpho-
25 linyl, thiamorpholinyl, furyl, thienyl, triazolyl, thiazolyl, β -carbolinyl, tetrazolyl, thiazolidinyl, benzofuanoyl, thiamorpholinyl sulfone, benzoxazolyl, oxopiperidinyl, oxopyrroldinyl, oxoazepinyl, azepinyl, isoxazolyl, tetrahydropyranyl, tetrahydrofuranyl, thiadiazolyl, thiadiazoyl, benzodioxolyl, thiophenyl, tetrahydro-thiophenyl, nicotinoyl, morpholine-
30 carbodithioyl and sulfolanyl.
35

- 10 -

The terms "HIV protease" and "HIV aspartyl protease" are used interchangeably and refer to the aspartyl protease encoded by the human immunodeficiency virus type 1 or 2. In a preferred embodiment of this invention, these terms refer to the human immunodeficiency virus type 1 aspartyl protease.

The term "pharmaceutically effective amount" refers to an amount effective in treating HIV infection in a patient. The term "prophylactically effective amount" refers to an amount effective in preventing HIV infection in a patient. As used herein, the term "patient" refers to a mammal, including a human.

The terms "pharmaceutically acceptable carrier or adjuvant" and "physiologically acceptable vehicle" refer to a non-toxic carrier or adjuvant that may be administered to a patient, together with a compound of this invention, and which does not destroy the pharmacological activity thereof.

As used herein, the compounds of this invention, including the compounds of formula I, are defined to include pharmaceutically acceptable derivatives thereof. A "pharmaceutically acceptable derivative" means any pharmaceutically acceptable salt, ester, or salt of such ester, of a compound of this invention or any other compound which, upon administration to a recipient, is capable of providing (directly or indirectly) a compound of this invention or an anti-virally active metabolite or residue thereof.

Salts derived from appropriate bases include alkali metal (e.g., sodium), alkaline earth metal (e.g., magnesium), ammonium and $N-(C_{1-4} \text{ alkyl})_4^+$ salts.

The term "unnatural alpha-amino acids" refers to alpha-amino acids which do not occur in nature but which can be derived from naturally occurring alpha-

- 11 -

amino acids or other chemical reagents by methods known to those skilled in the art.

The compounds of this invention contain one or more asymmetric carbon atoms and thus may occur as racemates and racemic mixtures, single enantiomers, diastereomeric mixtures and individual diastereomers. All such isomeric forms of these compounds are expressly included in the present invention. Each stereogenic carbon may be of the R or S configuration. Additionally, the compounds of the invention may be C₂-symmetric, wherein A = A', B = B', D = D', E = E' and G = G'.

Combinations of substituents and variables envisioned by this invention are only those that result in the formation of stable compounds. The term "stable", as used herein, refers to compounds which possess stability sufficient to allow manufacture and administration to a mammal by methods known in the art. Typically, such compounds are stable at a temperature of 40°C or less, in the absence of moisture or other chemically reactive conditions, for at least a week.

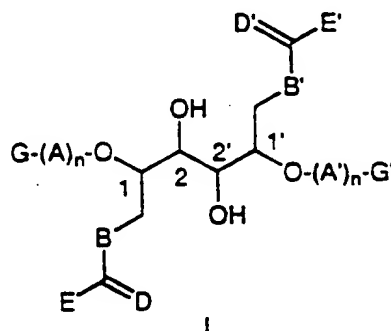
Pharmaceutically acceptable salts of the compounds of this invention include those derived from pharmaceutically acceptable inorganic and organic acids and bases. Examples of such acid salts include: acetate, adipate, alginate, aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, citrate, camphorate, camphorsulfonate, cyclopentanepropionate, digluconate, dodecylhydrogen-sulfate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptanoate, glycerophosphate, glycollate, hemisulfate, heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxy-ethanesulfonate, lactate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oxalate, pamoate, pectinate, perchlorate,

- 12 -

persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, salicylate, succinate, sulfate, tartrate, thiocyanate, tosylate and undecanoate.

This invention also envisions the
 5 quaternization of any basic nitrogen-containing groups of the compounds disclosed herein. The basic nitrogen can be quaternized with any agents known to those of ordinary skill in the art including, for example, lower alkyl halides, such as methyl, ethyl, propyl and butyl
 10 chloride, bromides and iodides; dialkyl sulfates including dimethyl, diethyl, dibutyl and diamyl sulfates; long chain halides such as decyl, lauryl, myristyl and stearyl chlorides, bromides and iodides; and aralkyl halides including benzyl and phenethyl
 15 bromides. Water or oil-soluble or dispersible products may be obtained by such quaternization.

The novel mannitol derivatives of this invention are those of formula I:



wherein:

20 each n is independently selected from the group consisting of 0, 1 and 2;

each A and A' is independently selected from the group consisting of a naturally occurring alpha-amino acid and an unnatural alpha-amino acid, wherein
 25 the amino group of each A or A' is bonded to G or G' or to the carboxy group of the adjacent residue A or A',

- 13 -

whichever is appropriate, and the carboxy group of A or A' is bonded to the amino group of the adjacent residue A or A' or to the oxygen of the structure, whichever is appropriate;

5 each G and G' is independently covalently attached to the amino group of the adjacent residue A or A' or to the oxygen of the structure (if the adjacent n = 0) and is selected from the group consisting of trityl; hydrogen; C₁-C₆ alkyl; R₃-CO-;
10 R₅(R₆R₇C)_mCO-; R₅(R₆R₇C)_mW-; R₈-W-; R₅(R₆R₇C)_m-P(O)(OR₉)-; R₈-P(O)(OR₉)-; and phthaloyl, which may be optionally substituted with one or more substituents R₄; wherein m = 1-3;

15 each W is independently selected from the group consisting of -OCO- and -SO₂-, with the proviso that W is not -SO₂- when W is attached to the oxygen of the structure;

20 each R₃ is independently selected from the group consisting of hydrogen; C₁-C₆ alkyl; C₂-C₆ alkenyl; phenyl; and naphthyl; said C₁-C₆ alkyl and C₂-C₆ alkenyl being optionally substituted with one or more substituents selected from the group consisting of hydroxyl, chlorine, and fluorine; and said phenyl and naphthyl being optionally substituted with one or more
25 substituents R₄;

 each R₄ is independently selected from the group consisting of C₁-C₄ alkyl; C₂-C₄ alkenyl; halogen; hydroxyl; nitro; C₁-C₃ alkoxy; and -CO-N(R₁₀)(R₁₀);

30 each R₅, R₆ and R₇ is independently selected from the group consisting of hydroxyl; hydrogen; chlorine; fluorine; C₁-C₃ alkoxy; a 5-7 membered heterocycle; C₁-C₃ alkyl; phenyl; and naphthyl; said C₁-C₃ alkyl being optionally substituted with one or more substituents selected from the group consisting of

- 14 -

chlorine, fluorine, and hydroxyl; and said phenyl and naphthyl being optionally substituted with one or more substituents R_4 ; or R_5 , R_6 and R_7 of a particular G or G' taken individually, or together in any combination, may be optionally joined to form a monocyclic, bicyclic, or tricyclic ring system, each ring of which is C_3 - C_6 cycloalkyl; with the proviso that R_5 , R_6 , or R_7 may not be chlorine, fluorine, or hydroxyl if it is present on the carbon adjacent to W;

each R_8 is independently selected from the group consisting of a 5-7 membered heterocycle; phenyl; and naphthyl; said phenyl and naphthyl being optionally substituted with one or more substituents R_4 ;

each R_9 is independently selected from the group consisting of C_1 - C_4 alkyl and phenyl;

each R_{10} is independently selected from the group consisting of hydrogen; C_1 - C_4 alkyl; and a 5-7 membered heterocycle;

each B and B' is independently selected from the group consisting of oxygen and sulfur;

each D and D' is independently selected from the group consisting of H_2 , oxygen and sulfur;

each E and E' is independently selected from the group consisting of Ar and $N(R_{11}R_{12})$;

each Ar is independently selected from the group consisting of phenyl and a 5-7 membered heterocycle; said phenyl and heterocycle being optionally substituted with one or more substituents R_4 (e.g., 3-pyridyl, 4-(1,2,3-thiadiazol)yl or 4-morpholine); and

each R_{11} and R_{12} is independently selected from the group consisting of C_1 - C_6 alkyl and C_2 - C_6 alkenyl.

- 15 -

Another embodiment of this invention includes compounds of formula I, as described above, wherein substituents n, A, A', G, G', R₃, R₄, R₅, R₆, R₇, m, W, R₈, R₉, R₁₀, B, B', D, D', E, E', R₁₁, R₁₂, and Ar are defined as above for formula I, with the proviso that when both n = 0, E and E' are not 3-pyridyl or diethylamino.

Except where expressly provided to the contrary, as used herein, the definitions of variables A, A', B, B', D, D', E, E', G, G', n, R₃-R₁₂, m, Ar and W are to be taken as they are defined immediately above.

An alternate embodiment of this invention includes compounds of formula I, wherein:

each n is independently selected from the group consisting of 0, 1 and 2;

each A and A' is independently selected from the group consisting of a naturally occurring alpha-amino acid and an unnatural alpha-amino acid, wherein the amino group of each A or A' is bonded to G or G' or to the carboxy group of the adjacent residue A or A', whichever is appropriate, and the carboxy group of A or A' is bonded to the amino group of the adjacent residue A or A' or to the oxygen of the structure, whichever is appropriate;

each G and G' is independently covalently attached to the amino group of the adjacent A or A' or to the oxygen of the structure (if the adjacent n = 0), and is selected from the group consisting of trityl; C₁-C₆ alkyl; R₃-CO-; R₅(R₆R₇C)_mCO-; R₅(R₆R₇C)_mW-; R₈-W-; R₅(R₆R₇C)_m-P(O)(OR₉)-; R₈-P(O)(OR₉)-; and phthaloyl, which may be optionally substituted with one or more substituents R₄; wherein m = 1-3;

each W is independently selected from the group consisting of -OCO- and -SO₂-, with the proviso

- 16 -

that W is not $-\text{SO}_2-$ when W is attached to the oxygen of the structure;

each R_3 is selected from the group consisting of hydrogen; C_1-C_6 alkyl; C_2-C_6 alkenyl; phenyl; and naphthyl; said C_1-C_6 alkyl and C_2-C_6 alkenyl being optionally substituted with one or more substituents selected from the group consisting of hydroxyl, chlorine, and fluorine; and said phenyl and naphthyl being optionally substituted with one or more substituents R_4 ;

each R_4 is independently selected from the group consisting of C_1-C_4 alkyl; C_2-C_4 alkenyl; halogen; hydroxyl; nitro; C_1-C_3 alkoxy; and $-\text{CO}-\text{N}(\text{R}_{10})(\text{R}_{10})$;

each R_5 , R_6 and R_7 is independently selected from the group consisting of hydroxyl; hydrogen; chlorine; fluorine; C_1-C_3 alkoxy; a 5-7 membered heterocycle; C_1-C_3 alkyl; phenyl; and naphthyl; said C_1-C_3 alkyl being optionally substituted with one or more substituents selected from the group consisting of chlorine, fluorine and hydroxyl; and said phenyl and naphthyl being optionally substituted with one or more substituents R_4 ; or R_5 , R_6 and R_7 of a particular G or G' taken individually, or together in any combination, may be optionally joined to form a monocyclic, bicyclic, or tricyclic ring system, each ring of which is C_3-C_6 cycloalkyl; with the proviso that R_5 , R_6 , and R_7 may not be chlorine, fluorine, or hydroxyl if it is present on the carbon adjacent to W;

each R_8 is independently selected from the group consisting of a 5-7 membered heterocycle; phenyl; and naphthyl; said phenyl and naphthyl being optionally substituted with one or more substituents R_4 ;

each R_9 is independently selected from the group consisting of C_1-C_4 alkyl and phenyl;

- 17 -

each R_{10} is independently selected from the group consisting of hydrogen; C_1-C_4 alkyl; and a 5-7 membered heterocycle;

5 each B and B' is independently selected from the group consisting of oxygen and sulfur;

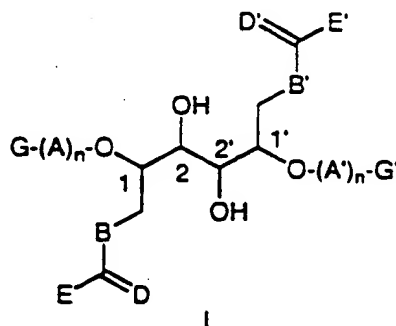
each D and D' is independently selected from the group consisting of H_2 , oxygen and sulfur;

each E and E' is independently selected from the group consisting of Ar and $N(R_{11}R_{12})$;

10 each Ar is independently selected from the group consisting of phenyl and a 5-7 membered heterocycle; said phenyl and heterocycle being optionally substituted with one or more substituents R_4 (e.g., 3-pyridyl, 4-(1,2,3-thiadiazol)yl or 4-morpholine); and

15 each R_{11} and R_{12} is independently selected from the group consisting of C_1-C_6 alkyl and C_2-C_6 alkenyl.

20 Another embodiment of this invention includes compounds of formula I:



25 wherein substituents n, A, A', G, G', R_3 , R_4 , R_5 , R_6 , R_7 , m, W, R_8 , R_9 , R_{10} , B, B', D, D', E, E', R_{11} , R_{12} , and Ar are defined as above for formula I, with the proviso that when both n = 0, E and E' are not 3-pyridyl or diethylamino.

Still another embodiment of this invention includes a subclass of compounds of formula I wherein:

- 18 -

each n is independently selected from the group consisting of 0, 1 and 2;

each A and A' is independently selected from the group consisting of a naturally occurring alpha-amino acid and an unnatural alpha-amino acid, wherein
5 the amino group of each A or A') is bonded to G or G' or to the carboxy group of the adjacent residue A or A', whichever is appropriate, and the carboxy group of A or A' is bonded to the amino group of the adjacent
10 residue A or A' or to the oxygen of the structure, whichever is appropriate;

each G and G' is independently covalently attached to the amino group of the adjacent residue A or A' or to the oxygen of the structure (if the
15 adjacent n = 0), and is selected from the group consisting of C₁-C₆ alkyl; R₃CO-; R₅(R₆R₇C)_mCO-; R₅(R₆R₇C)_mW-; and phthaloyl, which may be optionally substituted with one or more substituents R₄; wherein
m = 1-3;

each W is independently selected from the group consisting of -OCO- and -SO₂-, with the proviso
20 that W is not -SO₂- when W is attached to the oxygen of the structure;

each R₃ is independently selected from the group consisting of C₁-C₆ alkyl; phenyl; and naphthyl;
25 said C₁-C₆ alkyl being optionally substituted with one or more substituents selected from the group consisting of hydroxyl, chlorine, and fluorine; and said phenyl and naphthyl being optionally substituted with one or
30 more substituents R₄;

each R₄ is independently selected from the group consisting of C₁-C₄ alkyl; halogen; hydroxyl; nitro; and C₁-C₃ alkoxy;

- 19 -

each R_5 , R_6 and R_7 is optionally and independently selected from the group consisting of hydrogen; chlorine; fluorine; a 5-7 membered heterocycle; phenyl; naphthyl; and C_1 - C_3 alkyl, wherein
5 said C_1 - C_3 alkyl may be optionally substituted with one or more substituents selected from the group consisting of chlorine, fluorine, and hydroxyl; and wherein said phenyl and naphthyl may be optionally substituted with one or more substituents R_4 ; or R_5 , R_6 and R_7 of a
10 particular G or G' taken individually, or together in any combination, may be optionally joined to form a monocyclic, bicyclic, or tricyclic ring system, each ring of which is C_3 - C_6 cycloalkyl; with the proviso that R_5 , R_6 , and R_7 may not be chlorine, fluorine, or
15 hydroxyl if it is present on the carbon adjacent to W;
each B and B' is independently selected from the group consisting of oxygen and sulfur;
each D and D' is independently selected from the group consisting of H_2 ; oxygen and sulfur;
20 each E and E' is independently selected from the group consisting of Ar and $N(R_{11}R_{12})$;
each Ar is selected from the group consisting of phenyl and a 5-7 membered heterocycle; wherein said phenyl and 5-7 membered heterocycle may be optionally
25 substituted with one or more substituents R_4 ; and
each R_{11} and R_{12} is independently C_1 - C_6 alkyl.
According to still another embodiment of this invention, a subclass of compounds are those compounds of formula I wherein:
30 each n is independently selected from the group consisting of 0, 1 and 2;
each A and A' is independently selected from the group consisting of a naturally occurring alpha-amino acid and an unnatural alpha-amino acid, wherein
35 the amino group of each A or A' is bonded to G or G' or

- 20 -

to the carboxy group of the adjacent residue A or A', whichever is appropriate, and the carboxy group of A or A' is bonded to the amino group of the adjacent residue A or A' or to the oxygen of the structure, whichever is appropriate;

each G and G' is independently covalently attached to the amino group of the adjacent residue A or A' or to the oxygen of the structure (if the adjacent n = 0), and is selected from the group consisting of hydrogen; C₁-C₆ alkyl; R₃CO-; R₅(R₆R₇C)_mCO-; R₅(R₆R₇C)_mW-; and phthaloyl, which may be optionally substituted with one or more substituents R₄; wherein m = 1-3;

each W is independently selected from the group consisting of -OCO- and -SO₂-, with the proviso that W is not -SO₂- when W is attached to the oxygen of the structure;

each R₃ is independently selected from the group consisting of C₁-C₆ alkyl; phenyl; and naphthyl; said C₁-C₆ alkyl being optionally substituted with one or more substituents selected from the group consisting of hydroxyl, chlorine, and fluorine; and said phenyl and naphthyl being optionally substituted with one or more substituents R₄;

each R₄ is independently selected from the group consisting of C₁-C₄ alkyl; halogen; hydroxyl; nitro; and C₁-C₃ alkoxy;

each R₅, R₆, and R₇ is independently selected from the group consisting of hydrogen; chlorine; fluorine; C₁-C₃ alkyl; C₁-C₃ alkoxy; a 5-7 membered heterocycle; phenyl; and naphthyl; said C₁-C₃ alkyl being optionally substituted with one or more substituents selected from the group consisting of chlorine, fluorine, and hydroxyl; and said phenyl and

- 21 -

naphthyl being optionally substituted with one or more substituents R_4 ; or R_5 , R_6 , and R_7 of a particular G or G' taken individually, or together in any combination, may be optionally joined to form a monocyclic,
5 bicyclic, or tricyclic ring system, each ring of which is C_3 - C_6 cycloalkyl; with the proviso that R_5 , R_6 , or R_7 may not be chlorine, fluorine, or hydroxyl if it is present on the carbon adjacent to W;

each B and B' is independently selected from
10 the group consisting of oxygen and sulfur;

each D and D' is independently selected from the group consisting of H_2 ; oxygen and sulfur;

each E and E' is independently selected from the group consisting of Ar and $N(R_{11}R_{12})$, with the
15 proviso that each E and E' is not 3-pyridyl or diethylamino when both $n = 0$;

each Ar is independently selected from the group consisting of phenyl and a 5-7 membered heterocycle; wherein said phenyl and 5-7 membered
20 heterocycle may be optionally substituted with one or more substituents R_4 ; and

each R_{11} and R_{12} is independently C_1 - C_6 alkyl.

A subclass of compounds of this invention are those compounds of formula I, wherein:

25 each $n = 1$;

each A and A' is independently a naturally occurring alpha-amino acid, wherein the amino group of each A or A' is bonded to G or G' or to the carboxy group of the adjacent residue A or A', whichever is
30 appropriate, and the carboxy group of A or A' is bonded to the amino group of the adjacent residue A or A' or to the oxygen of the structure, whichever is appropriate;

each G and G' is independently covalently
35 attached to the amino group of the adjacent residue A

- 22 -

or A' or to the oxygen of the structure (if the adjacent $n = 0$), and is selected from the group consisting of hydrogen and $R_5(R_6R_7C)_mW-$, wherein W is -OCO- and $m = 1$;

5 each R_5 , R_6 , and R_7 is independently selected from the group consisting of hydrogen; chlorine; fluorine; C_1-C_3 alkyl; a 5-7 membered heterocycle; phenyl; and naphthyl; said C_1-C_3 alkyl being optionally substituted with one or more substituents selected from
10 the group consisting of chlorine, fluorine, and hydroxyl; and wherein said phenyl and naphthyl may be optionally substituted with one or more substituents R_4 ; or each R_5 , R_6 , and R_7 of a particular G or G' taken individually, or together in any combination, may be
15 optionally joined to form a monocyclic, bicyclic, or tricyclic ring system, each ring of which is C_3-C_6 cycloalkyl; with the proviso that R_5 , R_6 , or R_7 may not be chlorine, fluorine, or hydroxyl if it is present on the carbon adjacent to W;

20 each R_4 is independently selected from the group consisting of C_1-C_4 alkyl; halogen; hydroxyl; nitro; and C_1-C_3 alkoxy;

 each B and B' is independently selected from the group consisting of oxygen and sulfur;

25 each D and D' is independently selected from the group consisting of H_2 ; oxygen and sulfur;

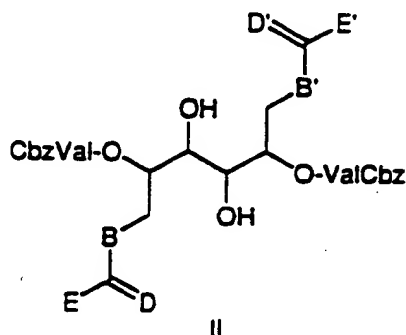
 each E and E' is independently selected from the group consisting of Ar and $N(R_{11}R_{12})$;

30 each Ar is independently selected from the group consisting of phenyl and a 5-7 membered heterocycle; wherein said phenyl and 5-7 membered heterocycle may be optionally substituted with one or more substituents R_4 ; and

 each R_{11} and R_{12} is independently C_1-C_6 alkyl.

- 23 -

A preferred subclass of compounds of this invention are those compounds of formula II:



wherein:

each B and B' is independently selected from the group consisting of oxygen and sulfur;

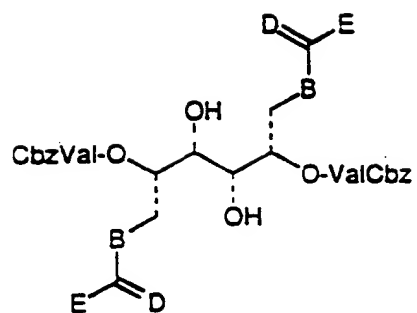
each D and D' is independently selected from the group consisting of H₂; oxygen and sulfur;

each E and E' is independently selected from the group consisting of Ar and NEt₂; and

each Ar is selected from the group consisting of phenyl; 3-hydroxyphenyl; 3-pyridyl; 4-(1,2,3-thiadiazol)-yl; and 4-morpholinyl.

Preferred mannitol derivatives of formula II of this invention are those in which (A)_n, (A')_n, G and G' are independently CbzVal; and groups B, D and E taken together are selected from the group consisting of: 1) benzoate; 2) 3-hydroxybenzoate; 3) nicotinoate; 4) 4-(1,2,3-thiadiazate); 5) benzyloxy; 6) thiobenzoate; 7) 4-morpholinecarbodithioate; and 8) N,N-diethylcarbodithioate. The preferred configuration of positions 1, 1', 2 and 2' is (S), (S), (R) and (R), respectively, and are derived from the starting material, such as from L-mannitol. Such preferred compounds include those depicted in Table I.

TABLE 1



Compound	B	D	E
1	O	O	phenyl
2	O	O	3-hydroxyphenyl
3	O	O	3-pyridyl
4	O	O	4-(1,2,3-thiadiazol)-yl
5	O	H ₂	phenyl
6	S	O	phenyl
7	S	S	4-morpholinyl
8	S	S	diethylamino

- 25 -

It should be understood that for all compounds of formula I and formula II, as defined herein, B, D, and E taken together may be selected from the group consisting of benzoate; 3-hydroxybenzoate; 5 nicotinoate; 4-(1,2,3-thiadiazate); benzyloxy; thiobenzoate; 4-morpholinecarbodithioate; and N,N-diethylcarbodithioate. In addition, A and G taken together may form carbobenzyloxyvaline.

Preferred compounds of this invention are:

10 1,6-Di-O-benzoyl-2,5-di-O-(N-carbobenzyloxyvalyl)-L-mannitol (compound 1);

1,6-Di-O-(3-hydroxy)benzoyl-2,5-di-O-(N-carbobenzyloxyvalyl)-L-mannitol (compound 2);

15 1,6-Di-O-nicotinoyl-2,5-di-O-(N-carbobenzyloxyvalyl)-L-mannitol (compound 3);

1,6-Di-O-[4-(1,2,3-thiadiazol)-yl]-2,5-di-O-(N-carbobenzyloxyvalyl)-L-mannitol; (compound 4);

1,6-Di-O-benzyl-2,5-di-O-(N-carbobenzyloxyvalyl)-L-mannitol (compound 5);

20 1,6-Di-S-benzoyl-2,5-di-O-(N-carbobenzyloxyvalyl)-L-mannitol (compound 6);

1,6-Di-S-(4-morpholinothiocarbonyl)-2,5-di-O-(N-carbobenzyloxyvalyl)-L-mannitol (compound 7); and

25 1,6-Di-S-(N,N-diethylthiocarbonyl)-2,5-di-O-(N-carbobenzyloxyvalyl)-L-mannitol (compound 8).

The compounds of this invention may be synthesized using conventional techniques. Advantageously, these compounds are conveniently synthesized from readily available starting materials.

30 The compounds of this invention are among the most readily synthesized HIV protease inhibitors known. Previously described HIV protease inhibitors often contain more than six chiral centers, numerous peptide linkages and/or require air-sensitive reagents (such as
35 organometallic complexes) to effect their syntheses.

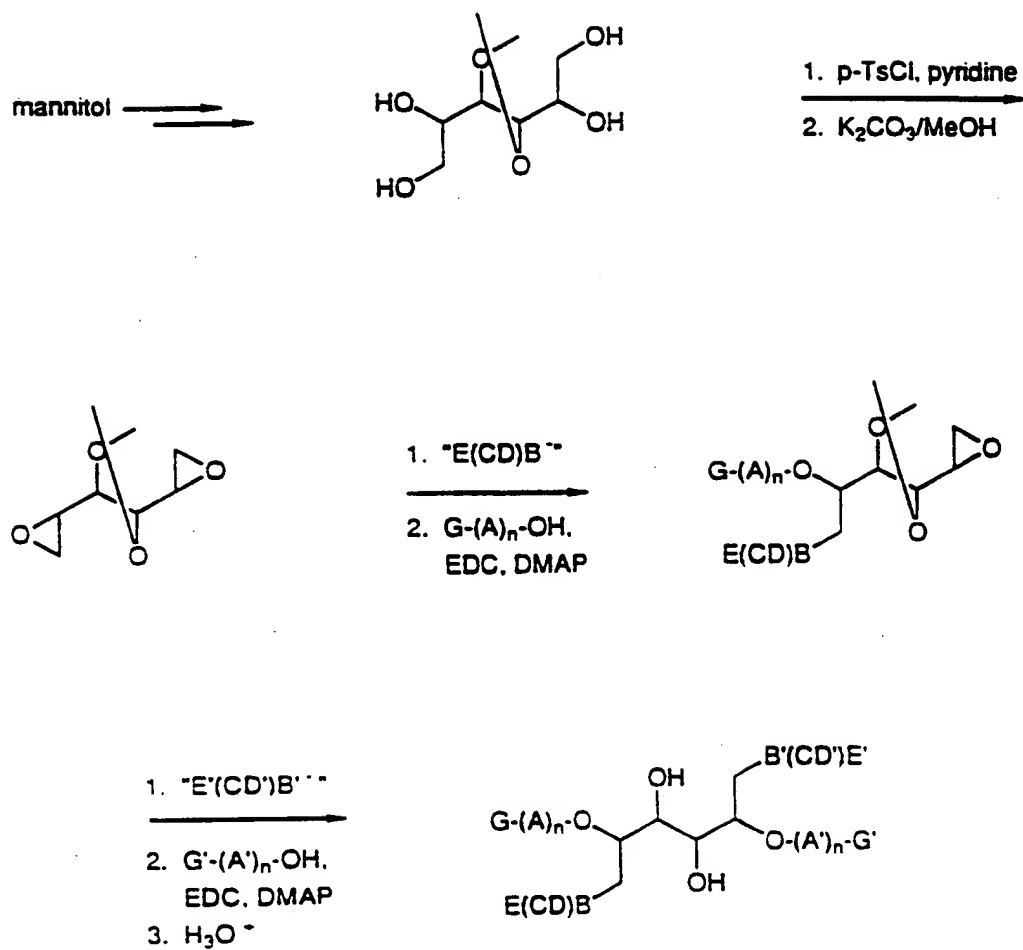
- 26 -

The relative ease with which the compounds of this invention can be synthesized represents an enormous advantage in the large scale production of these compounds.

5 In general, mannitol derivatives of formula I and formula II are conveniently obtained from D- or L-mannitol. Such enantiomeric or diastereomeric mannitol derivatives may be conveniently prepared from commercially available D-or L-mannitol using known
10 techniques. Although this invention envisions the use of either D- or L-mannitol, or racemic mixtures thereof, as the scaffold upon which to append various functional groups, L-mannitol is preferred.

 Using standard techniques, D- or L-mannitol
15 may be derivatized according to Scheme I, as depicted below:

- 27 -



Scheme 1

- 28 -

D- or L-mannitol may be converted to its monoketal using acetone or other ketones known to those skilled in the art (T.W. Greene, Protective Groups in Organic Synthesis, John Wiley and Sons (1991) and references cited therein). The ketal may then be converted to its bis epoxide, first by selective tosylation of the primary hydroxyl groups and then by treatment with potassium carbonate base to effect bis epoxidation with concomitant departure of tosylate. An epoxide functionality may then be opened at its least hindered carbon atom with a nucleophile having the general formula $E(CD)B$, wherein E, D and B are defined as above for the compounds of formula I, giving the monoaddition product (alcohol epoxide). The resulting hydroxyl group may then be esterified in the presence of EDC (1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride) and DMAP (4-dimethylaminopyridine) with an N-protected alpha-amino acid having the general formula $G-(A)_n-OH$, wherein G, A, and n are defined as above for the compounds of formula I, to give an alpha-amino acid-functionalized epoxide. The remaining epoxide may then be opened with a nucleophile having the general formula $E'(CD')B'$, wherein E', D' and B' are defined as above for the compounds of formula I, the resulting diol esterified with $G'-(A')_n-OH$, wherein G' and A' are defined as above for the compounds of formula I, and the ketal protecting group removed with aqueous acid to give compounds of the general formula I.

For examples of methods to obtain enantiomeric or diastereomeric D- or L-mannitol derivatives by the procedures illustrated in Scheme 1, see L.F. Wiggins, J. Chem. Soc., p. 13 (1946); Y. Le Merrer et al., Tetrahedron Lett., 26, pp. 319-22 (1985) and Y. Le Merrer et al., Heterocycles, 25, pp.

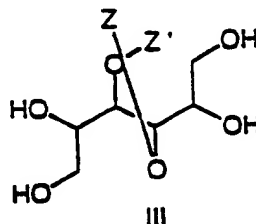
- 29 -

541-48 (1987). Alternatively, enantiomeric or diastereomeric glycols may be obtained from the oxidation of a suitable olefin-containing precursor with, for example, osmium tetroxide, which may optionally be used in conjunction with another oxidizing agent, such as N-ethylmorpholine N-oxide (see, van Rheenen et al., Tetrahedron Lett., pp. 1973-76 (1976) and Evans and Kaldor, J. Org. Chem., 55, pp. 1698-1700 (1990)). The osmium tetroxide-promoted oxidation may also be carried out in the presence of various chiral ligands to promote asymmetric (i.e., face-selective) oxidation of the olefin (Wai et al., J. Am. Chem. Soc., 111, p. 1123 (1989)). Another method for constructing the glycol nucleus includes the reductive coupling of two appropriately derivatized aldehydes, which may be the same or different, in a pinacol reaction. This reaction can be carried out using low valent metals, including titanium (J.E. McMurry, Acc. Chem. Res., 16, pp. 405-411 (1983)) and vanadium (Freudenberger et al., J. Am. Chem. Soc., 111, pp. 8014-16 (1989)), and by various lanthanide iodides (Namy et al., Tetrahedron Lett., 24, pp. 765-66 (1983) and Imamoto et al., Tetrahedron Lett., 23, pp. 1353-56 (1982)).

Compounds of the present invention may be synthesized using known techniques. D- or L-mannitol may be converted to the ketal of formula III (shown below) using acid catalysts known to those skilled in the art and carbonyl compounds of the type $ZC(O)Z'$; wherein Z and Z' are independently selected from the group consisting of hydrogen, C_1 - C_{20} alkyl, C_3 - C_8 cycloalkyl, phenyl, naphthyl, and a 5-7 membered heterocycle; wherein said C_1 - C_{20} alkyl, C_3 - C_8 cycloalkyl, phenyl, naphthyl, and 5-7 membered heterocycle groups may be optionally substituted with

- 30 -

one or more substituents selected from the group consisting of halogen, hydroxyl, alkoxyl, phenoxyl, nitro, carboxylate and sulfonate; and wherein Z and Z' may optionally be joined to form a cyclic ketone.

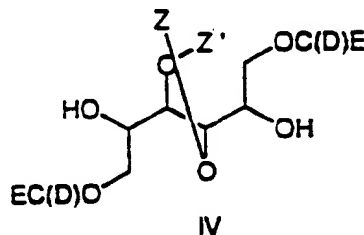


5 Such ketones may be obtained commercially or may be synthesized by known methods. Acetone is especially preferred, using sulfuric acid as the reaction catalyst.

10 The ketal of formula III may then be selectively esterified at the primary hydroxyl positions with carboxylic acids, or acid derivatives thereof, having the general formula E-C(D)-X, wherein E and D are defined as above for the compounds of formula I and X is a leaving group which suitably activates the
15 C=D carbonyl. Such leaving groups are well known in the art and include halides, hydroxide, alkoxides, phenoxides, and sulfonates. The esterification may optionally be run in the presence of an organic base, such as triethylamine, diisopropylamine, ethyldiisopropylamine, pyridine, 4-dimethylaminopyridine, or
20 mixtures thereof. Preferred leaving groups include the halides, and chlorine is especially preferred. Preferred organic bases include pyridine and 4-dimethylaminopyridine, and especially preferred are
25 mixtures thereof. In a preferred embodiment of the invention, E-C(D) taken together is benzoate. In a second preferred embodiment, E-C(D) is 3-hydroxybenzoate. In a third preferred embodiment, E-C(D) is

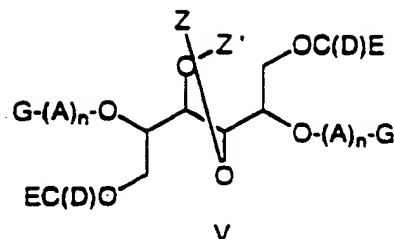
- 31 -

nicotinoate. In a fourth preferred embodiment, E-C(D) is 1,2,3-thiadiazole-4-carboxylate. The esterification of the compound of formula III with E-C(D)-X leads to compounds of the formula IV:



5 wherein Z and Z' are defined as above for
 formula III. The diol of formula IV may then be
 further esterified with compounds having the general
 formula $G-(A)_n-X$, wherein G, A and n are defined as
 above for the compounds of formula I, and X is a
 10 leaving group which enhances the electrophilicity of G
 (when $n = 0$), or A. Suitable leaving groups are well
 known in the art and include but are not limited to
 halides, hydroxyl, alkoxides, phenoxides, and
 sulfonates. The esterification may optionally proceed
 15 in the presence of a reaction catalyst such as 4-
 dimethylaminopyridine, 1-(3-dimethylaminopropyl)-3-
 ethylcarbodiimide hydrochloride (EDC), dicyclohexyl-
 carbodiimide (DCC), diisopropylcarbodiimide (DIC), and
 mixtures thereof. In a preferred embodiment of the
 20 invention, the leaving group is hydroxyl and the
 esterification catalyst is a mixture of EDC and
 4-dimethylaminopyridine. In an especially preferred
 embodiment of the invention, G = benzyloxycarbonyl
 (Cbz), A = valine (Val) and $n = 1$. The esterification
 25 of the compound of formula IV with $G-(A)_n-X$ leads to
 compounds of the formula V:

- 32 -

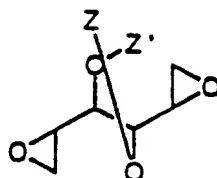


wherein Z, Z', E, C, and D are defined as above for formula IV. The compound of formula V may then be treated with water and acid to remove the ketal protecting group and yield mannitol derivatives of formula I. Suitable acids used to remove ketal protecting groups are well known to those skilled in the art and include but are not limited to hydrochloric, sulfuric, acetic, p-toluenesulfonic and trifluoroacetic acid. A preferred acid is trifluoroacetic acid.

Alternatively, compounds of the present invention may be obtained directly from D- or L-mannose. In a preferred embodiment of the invention, L-mannose is the chosen enantiomer. Mannose may be reduced to mannitol by the action of a hydride donor. Hydride donors are well known to those skilled in the art and include but are not limited to sodium borohydride, sodium cyanoborohydride, lithium aluminium hydride, lithium tri-sec-butylborohydride and potassium tri-sec-butylborohydride. The reduction of mannose to mannitol may take place in a common solvent such as water, methanol, ethanol, 1-propanol, 2-propanol, tetrahydrofuran and mixtures thereof. In a preferred embodiment of the invention, the hydride donor is sodium borohydride and the solvent is methanol.

- 33 -

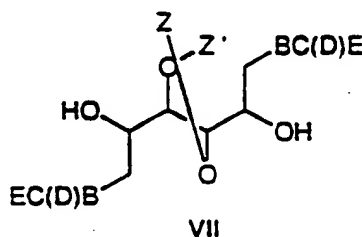
Mannitol may then be converted to the ketal of formula III by methods described above. The ketal of formula III may then be selectively condensed at the primary hydroxyl positions with an electrophile which, after forming a stable covalent bond with oxygen, can itself serve as a leaving group. Such electrophiles are known to those skilled in the art and include but are not limited to acid halide and acid anhydride derivatives of p-toluenesulfonic acid, 4-bromobenzenesulfonic acid, methanesulfonic acid and trifluoromethanesulfonic acid. Preferred is p-toluenesulfonyl chloride. The resulting bis adduct may be treated with base in a common solvent (defined above) to effect intramolecular bis-epoxidation via attack of the remaining hydroxyl groups at the terminal carbons with concomitant departure of the electrophile-oxygen leaving group. Such bases are well known to those skilled in the art and include, but are not limited to metal hydroxides such as sodium hydroxide, potassium hydroxide and lithium hydroxide, metal carbonates such as sodium carbonate, potassium carbonate and lithium carbonate, and metal hydrogen carbonates such as sodium bicarbonate, potassium bicarbonate and lithium bicarbonate. In a preferred embodiment of the invention, the base is potassium carbonate and the common solvent is methanol. Bis epoxides of this type are represented below in formula VI:



VI

- 34 -

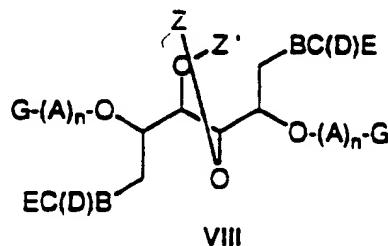
wherein Z and Z' are defined as above for formula III. The bis epoxide of formula VI may be alkylated at the least hindered carbon atoms with a nucleophile having the formula E-C(D)-BH, wherein E, D and B are defined as above for the compounds of formula I, to give a diol. In a preferred embodiment of the invention, E-C(D)-BH is benzyl alcohol. In a second preferred embodiment, E-C(D)-BH is thiobenzoic acid. In a third preferred embodiment, E-C(D)-BH is 4-morpholinecarbodithioic acid. Diols obtained by this method are represented below in formula VII:



wherein Z, Z', E, C, and B are defined as above for formula IV. The diol of formula VII may then be further esterified with compounds having the general formula G-(A)_n-X, wherein G, A and n are defined as above for the compounds of formula I, and X is a leaving group which enhances the electrophilicity of G (when n = 0), or A. Suitable leaving groups are well known in the art and include but are not limited to halides, hydroxyl, alkoxides, phenoxides, and sulfonates. The esterification may optionally proceed in the presence of a reaction catalyst, such as 4-dimethylamion-pyridine, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), dicyclohexylcarbodiimide (DCC), diisopropylcarbodiimide (DIC), and mixtures thereof. In a preferred embodiment of the invention, the leaving group is hydroxyl and the

- 35 -

esterification catalyst is a mixture of EDC and 4-dimethylamino-pyridine. In an especially preferred embodiment of this invention, G = benzoyloxycarbonyl (Cbz), A = valine (Val) and n = 1. The esterification of the compound of formula IV with G-(A)_n-X leads to compounds of the formula VIII:

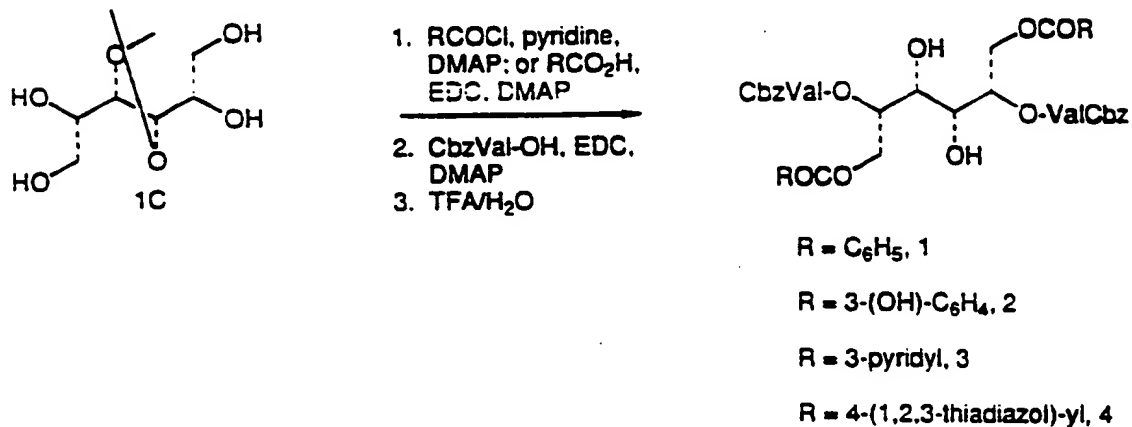


wherein Z, Z', E, C, B, G, A, and n are defined as above for formula V. The compound of formula VIII may then be treated in a similar fashion as the compound of formula V, wherein the ketal protecting group is liberated to afford mannitol derivatives of formula I. In a preferred embodiment of the invention, the acid used in conjunction with water is trifluoroacetic acid.

Mannitol derivatives of the preferred embodiment of the invention were synthesized from 3,4-O-isopropylidene-L-mannitol (1C), which was derived from L-mannitol (see Schemes 2-3 below), according to procedures described by L.F. Wiggins, J. Chem. Soc., p. 13, (1946); Le Merrer et al., Tetrahedron Lett., 26, pp. 319-22 (1985) and Le Merrer et al., Heterocycles, 25, pp. 541-48 (1987), wherein D-mannitol was used. The bis-ester core series of L-mannitol derivatives 1-4 were obtained by selective acylation of both primary hydroxyl groups of compound 1C with an either a carboxylic acid halide in the presence of pyridine, or a carboxylic acid in the presence of EDC and DMAP, followed by coupling the resulting diol with

- 36 -

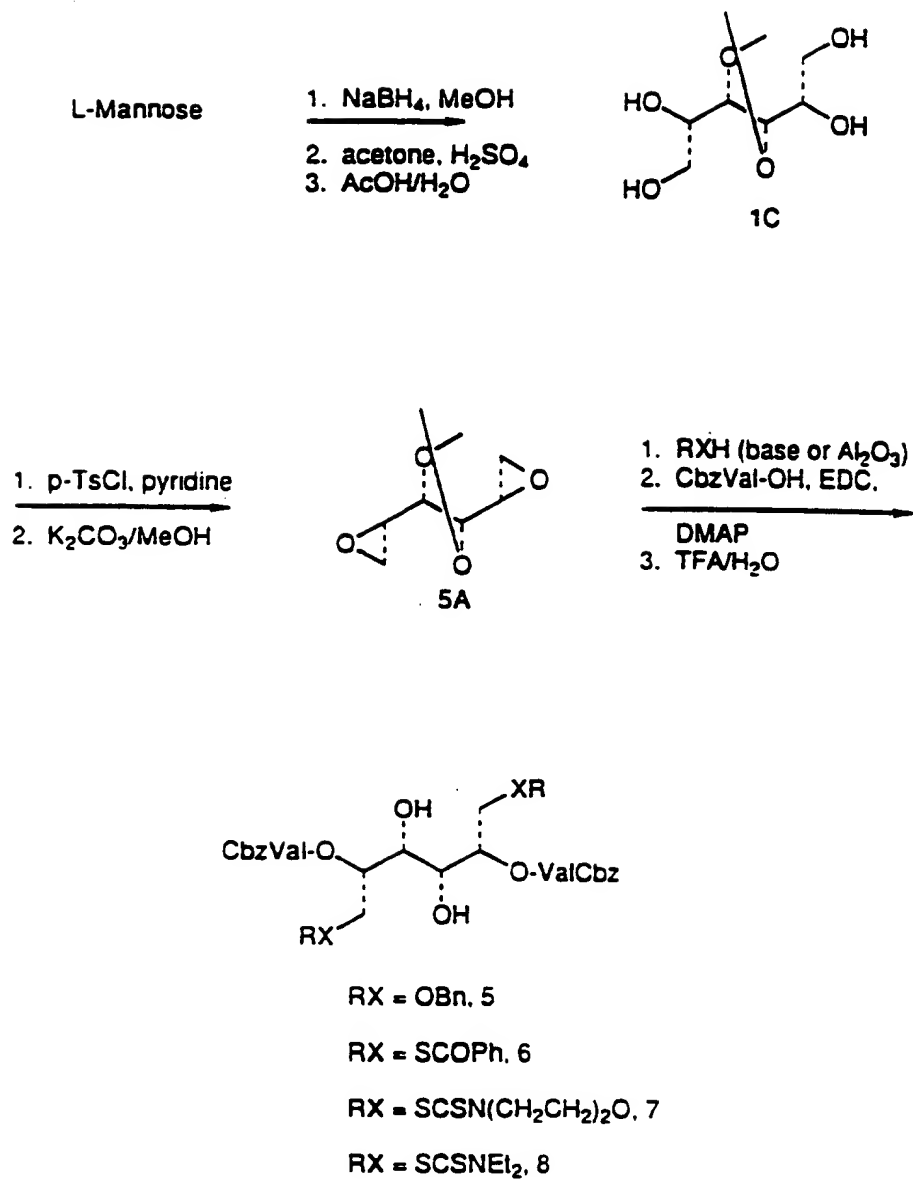
carbobenzyloxy-L-valine (CbzVal-OH) in the presence of EDC and DMAP. Subsequent treatment with aqueous trifluoroacetic acid to remove the acetonide protecting group yielded L-mannitol derivatives 1-4 (Scheme 2).



Scheme 2

5 L-mannitol derivatives 5-8 were similarly
 derived from compound 1C, which was obtained from the
 sodium borohydride reduction of L-mannose, followed by
 tris ketalization with acetone and sulfuric acid, and
 subsequent removal of the terminal ketals with acetic
 10 acid and water (Scheme 3).

- 37 -



Scheme 3

- 38 -

Acetonide 1C was then selectively tosylated at each primary hydroxyl site with p-toluenesulfonyl chloride in pyridine, and then treated with potassium carbonate in methanol to afford bis epoxide 5A (see, L.F. Wiggins, J. Chem. Soc., p. 13, (1946); Le Merrer et al., Tetrahedron Lett., 26, 319 (1985) and Le Merrer et al., Heterocycles, 25, p. 541 (1987)). Mannitol derivatives 5 and 6 were obtained by treating bis epoxide 5A with either an alcohol or a thiocarboxylic acid using aluminum oxide as a catalyst (see, Posner et al., Tetrahedron Lett., 42, p. 3596 (1975) and Posner et al., J. Am. Chem. Soc., 99, pp. 25 and 8208 (1977)), esterifying the resulting diols with CbzVal-OH in the presence of EDC and DMAP, and removing the acetonide protecting group with TFA/water. Mannitol derivatives 7 and 8 were obtained by treating bis epoxide 5A with a dithioic acid salt, in the presence dimethylformamide solvent (for an analogous reaction wherein an N-acylaziridine is ring-opened with mercaptan nucleophiles, see Kempf et al., European patent application 402646 A1). The dithioic acid salt may be used directly or generated in situ from reaction with an amine base. The resulting diols were then esterified with CbzVal-OH in the presence of EDC and DMAP, and the acetonide protecting group was removed with TFA and water.

As can be appreciated by the skilled artisan, the above synthetic schemes are not intended to comprise a comprehensive list of all means by which the compounds described and claimed in this application may be synthesized. Further methods will be evident to those of ordinary skill in the art.

The compounds of this invention may be modified by appending appropriate functionalities to enhance selective biological properties. Such

- 39 -

modifications are known in the art and include those which increase biological penetration into a given biological system (e.g., blood, lymphatic system, central nervous system), increase oral availability, increase solubility to allow administration by injection, alter metabolism and alter rate of excretion.

As discussed above, the novel compounds of the present invention are excellent ligands for aspartyl proteases, particularly HIV-1, HIV-2 and HTLV-1 proteases. Accordingly, these compounds are capable of targeting and inhibiting late stage events in HIV replication, i.e., the processing of the viral polyproteins by HIV encoded proteases. Compounds according to this invention advantageously inhibit the ability of the HIV-1 virus to infect immortalized human T cells over a period of days, as determined by an assay of extracellular p24 antigen -- a specific marker of viral replication (see, Meek et al., Nature, 343, pp. 90-92 (1990)).

In addition to their use in the prophylaxis or treatment of HIV or HTLV infection, the compounds according to this invention may also be used as inhibitory or interruptive agents for other viruses which depend on aspartyl proteases, similar to HIV or HTLV aspartyl proteases, for obligatory events in their life cycle. Such compounds inhibit the proteolytic processing of viral polyprotein precursors by inhibiting aspartyl protease. Because aspartyl protease is essential for the production of mature virions, inhibition of that processing effectively blocks the spread of virus by inhibiting the production and reproduction of infectious virions, particularly from chronically infected cells. The compounds of this invention advantageously inhibit enzymatic activity of

- 40 -

aspartyl proteases and inhibit the ability of aspartyl proteases to catalyze the hydrolysis of peptide bonds.

The compounds of this invention may be employed in a conventional manner for the treatment or prevention of HIV, HTLV, and other viruses which depend on aspartyl proteases for obligatory events in their life cycle. Such methods of treatment, their dosage levels and requirements may be selected by those of ordinary skill in the art from available methods and techniques. For example, a compound of this invention may be combined with a pharmaceutically acceptable adjuvant for administration to a virally-infected patient in a pharmaceutically acceptable manner and in an amount effective to lessen the severity of the viral infection.

Alternatively, the compounds of this invention may be used in vaccines and methods for protecting individuals against viral infection over an extended period of time. The compounds may be employed in such vaccines either alone or together with other compounds of this invention in a manner consistent with the conventional utilization of protease inhibitors in vaccines. For example, a compound of this invention may be combined with pharmaceutically acceptable adjuvants conventionally employed in vaccines and administered in prophylactically effective amounts to protect individuals over an extended period time against viral infections, such as HIV infection. As such, the novel protease inhibitors of this invention can be administered as agents for treating or preventing viral infections, including HIV infection, in a mammal.

The compounds of this invention may be administered to a healthy or HIV-infected patient either as a single agent or in combination with other

- 41 -

anti-viral agents which interfere with the replication cycle of HIV. By administering the compounds of this invention with other anti-viral agents which target different events in the viral life cycle, the therapeutic effect of these compounds is potentiated. For instance, the co-administered anti-viral agent can be one which targets early events in the life cycle of the virus, such as cell entry, reverse transcription and viral DNA integration into cellular DNA. Anti-HIV agents targeting such early life cycle events include, didanosine (ddI), alcitabine (ddC), d4T, zidovudine (AZT), polysulfated polysaccharides, sT4 (soluble CD4) -- which blocks attachment or adsorption of the virus to host cells -- and other compounds which block binding of virus to CD4 receptors on CD4-bearing T-lymphocytes. Other retroviral reverse transcriptase inhibitors, such as derivatives of AZT, may also be co-administered with the compounds of this invention to provide therapeutic treatment for substantially reducing or eliminating viral infectivity and the symptoms associated therewith. Examples of other anti-viral agents include ganciclovir, dideoxycytidine, trisodium phosphonoformate, eflornithine, ribavirin, acyclovir, alpha interferon and trimenotrexate. Additionally, non-nucleoside inhibitors of reverse transcriptase, such as TIBO or nevirapine, may be used to potentiate the effect of the compounds of this invention, as may viral uncoating inhibitors, inhibitors of trans-activating proteins such as tat or rev, or inhibitors of the viral integrase. These compounds may also be co-administered with other inhibitors of HIV aspartyl protease.

Combination therapies according to this invention exert a synergistic effect in inhibiting HIV replication because each component agent of the

- 42 -

combination acts on a different site of HIV replication. The use of such combinations also advantageously reduces the dosage of a given conventional anti-retroviral agent which would be
5 required for a desired therapeutic or prophylactic effect as compared to when that agent is administered as a monotherapy. These combinations may reduce or eliminate the side effects of conventional single anti-retroviral agent therapies while not interfering with
10 the anti-retroviral activity of those agents. These combinations reduce potential of resistance to single agent therapies, while minimizing any associated toxicity. These combinations may also increase the efficacy of the conventional agent without increasing
15 the associated toxicity. In particular, we have discovered that these compounds act synergistically in preventing the replication of HIV in human T cells. Preferred combination therapies include the administration of a compound of this invention with
20 AZT, ddI, ddC or d4T.

Alternatively, the compounds of this invention may also be co-administered with other HIV protease inhibitors such as Ro 31-8959 (Roche), L-735,524 (Merck), XM 323 (Du-Pont Merck) and A-80,987
25 (Abbott) to increase the effect of therapy or prophylaxis against various viral mutants or members of other HIV quasi species.

We prefer administering the compounds of this invention as single agents or in combination with
30 retroviral reverse transcriptase inhibitors, such as derivatives of AZT, or other HIV aspartyl protease inhibitors. We believe that the co-administration of the compounds of this invention with retroviral reverse transcriptase inhibitors or HIV aspartyl protease
35 inhibitors may exert a substantial synergistic effect,

- 43 -

thereby preventing, substantially reducing, or completely eliminating viral infectivity and its associated symptoms.

5 The compounds of this invention can also be administered in combination with immunomodulators (e.g., bropirimine, anti-human alpha interferon antibody, IL-2, GM-CSF, methionine enkephalin, interferon alpha, diethyldithiocarbamate, tumor necrosis factor, naltrexone and rEPO); antibiotics
10 (e.g., pentamidine isethionate) or vaccines to prevent or combat infection and disease associated with HIV infection, such as AIDS and ARC.

15 When the compounds of this invention are administered in combination therapies with other agents, they may be administered sequentially or concurrently to the patient. Alternatively, pharmaceutical or prophylactic compositions according to this invention may be comprised of a combination of an aspartyl protease inhibitor of this invention and
20 another therapeutic or prophylactic agent.

Although this invention focuses on the use of the compounds disclosed herein for preventing and treating HIV infection, the compounds of this invention can also be used as inhibitory agents for other viruses
25 which depend on similar aspartyl proteases for obligatory events in their life cycle. These viruses include, but are not limited to, other AIDS-like diseases caused by retroviruses, such as simian immunodeficiency viruses, HTLV-I and HTLV-II. In
30 addition, the compounds of this invention may also be used to inhibit other aspartyl proteases, and in particular, other human aspartyl proteases, including renin and aspartyl proteases that process endothelin precursors.

- 44 -

Pharmaceutical compositions of this invention comprise any of the compounds of the present invention, and pharmaceutically acceptable salts thereof, with any pharmaceutically acceptable carrier, adjuvant or vehicle. Pharmaceutically acceptable carriers, adjuvants and vehicles that may be used in the pharmaceutical compositions of this invention include, but are not limited to, ion exchangers, alumina, aluminum stearate, lecithin, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, polyethylene glycol and wool fat.

The pharmaceutical compositions of this invention may be administered orally, parenterally, by inhalation spray, topically, rectally, nasally, buccally, vaginally or via an implanted reservoir. We prefer oral administration or administration by injection. The pharmaceutical compositions of this invention may contain any conventional non-toxic pharmaceutically-acceptable carriers, adjuvants or vehicles. The term "parenteral" as used herein includes subcutaneous, intracutaneous, intravenous, intramuscular, intra-articular, intrasynovial, intrasternal, intrathecal, intralesional and intracranial injection or infusion techniques.

The pharmaceutical compositions may be in the form of a sterile injectable preparation, for example,

- 45 -

as a sterile injectable aqueous or oleaginous suspension. This suspension may be formulated according to techniques known in the art using suitable dispersing or wetting agents (such as, for example, Tween 80) and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are mannitol, water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil may be employed, including synthetic mono- or diglycerides. Fatty acids, such as oleic acid and its glyceride derivatives are useful in the preparation of injectables, as are natural pharmaceutically-acceptable oils, such as olive oil or castor oil, especially in their polyoxyethylated versions. These oil solutions or suspensions may also contain a long-chain alcohol diluent or dispersant, such as Ph. Helv or a similar alcohol.

The pharmaceutical compositions of this invention may be orally administered in any orally acceptable dosage form including, but not limited to, capsules, tablets, and aqueous suspensions and solutions. In the case of tablets for oral use, carriers which are commonly used include lactose and corn starch. Lubricating agents, such as magnesium stearate, are also typically added. For oral administration in a capsule form, useful diluents include lactose and dried corn starch. When aqueous suspensions are administered orally, the active ingredient is combined with emulsifying and suspending

- 46 -

agents. If desired, certain sweetening and/or flavoring and/or coloring agents may be added.

5 The pharmaceutical compositions of this invention may also be administered in the form of suppositories for rectal administration. These compositions can be prepared by mixing a compound of this invention with a suitable non-irritating excipient which is solid at room temperature but liquid at the rectal temperature and therefore will melt in the
10 rectum to release the active components. Such materials include, but are not limited to, cocoa butter, beeswax and polyethylene glycols.

Topical administration of the pharmaceutical compositions of this invention is especially useful
15 when the desired treatment involves areas or organs readily accessible by topical application. For application topically to the skin, the pharmaceutical composition should be formulated with a suitable ointment containing the active components suspended or
20 dissolved in a carrier. Carriers for topical administration of the compounds of this invention include, but are not limited to, mineral oil, liquid petroleum, white petroleum, propylene glycol, polyoxyethylene polyoxypropylene compound, emulsifying wax and
25 water. Alternatively, the pharmaceutical compositions can be formulated with a suitable lotion or cream containing the active compound suspended or dissolved in a carrier. Suitable carriers include, but are not limited to, mineral oil, sorbitan monostearate,
30 polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water. The pharmaceutical compositions of this invention may also be topically applied to the lower intestinal tract by rectal suppository formulation or in a suitable enema
35 formulation. Topically-transdermal patches are also included in this invention.

- 47 -

The pharmaceutical compositions of this invention may be administered by nasal aerosol or inhalation. Such compositions are prepared according to techniques well-known in the art of pharmaceutical formulation and may be prepared as solutions in saline, employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, fluorocarbons, and/or other solubilizing or dispersing agents known in the art.

Dosage levels of between about .01 and about 25 mg/kg body weight per day, preferably between about 0.5 and about 25 mg/kg body weight per day of the active ingredient compound are useful in the prevention and treatment of viral infection, including HIV infection. Typically, the pharmaceutical compositions of this invention will be administered from about 1 to about 5 times per day or alternatively, as a continuous infusion. Such administration can be used as a chronic or acute therapy. The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary depending upon the patient treated and the particular mode of administration. A typical preparation will contain from about 5% to about 95% active compound (w/w). Preferably, such preparations contain from about 20% to about 80% active compound.

Upon improvement of a patient's condition, a maintenance dose of a compound, composition or combination of this invention may be administered, if necessary. Subsequently, the dosage or frequency of administration, or both, may be reduced, as a function of the symptoms, to a level at which the improved condition is retained. When the symptoms have been alleviated to the desired level, treatment should cease. Patients may, however, require intermittent

- 48 -

treatment on a long-term basis, upon any recurrence of disease symptoms.

As the skilled artisan will appreciate, lower or higher doses than those recited above may be required. Specific dosage and treatment regimens for any particular patient will depend upon a variety of factors, including the activity of the specific compound employed, the age, body weight, general health status, sex, diet, time of administration, rate of excretion, drug combination, the severity and course of the infection, the patient's disposition to the infection and the judgment of the treating physician.

The compounds of this invention are also useful as commercial reagents which effectively bind to aspartyl proteases, particularly HIV aspartyl protease. As commercial reagents, the compounds of this invention, and their derivatives, may be used to block proteolysis of a target peptide, such as an aspartyl protease, or may be derivatized to bind to a stable resin as a tethered substrate for affinity chromatography applications. These and other uses which characterize commercial aspartyl protease inhibitors will be evident to those of ordinary skill in the art.

This invention also includes methods for synthesizing aspartyl protease inhibitors. One method comprises the steps of:

(a) treating mannitol with a carbonyl compound in the presence of an acid to yield a ketal derivative; said carbonyl compound being selected from the group consisting of acetone, cyclopentanone, cyclohexanone, and benzaldehyde; and said acid being selected from the group consisting of sulfuric, hydrochloric, acetic, trifluoroacetic, and mixtures thereof;

- 49 -

(b) esterifying said ketal derivative at each primary hydroxyl site thereof with a sulfonyl chloride to yield a bis sulfonate ester; said sulfonyl chloride being selected from the group consisting of
5 p-toluenesulfonyl chloride, 4-bromobenzenesulfonyl chloride, methanesulfonyl chloride, and trifluoromethanesulfonyl chloride;

(c) reacting said bis sulfonate ester with a base to yield a bis epoxide; said base being
10 selected from the group consisting of metal hydroxides, metal carbonates, and metal bicarbonates; and said bis epoxide having two least hindered sites;

(d) opening said bis epoxide at each least hindered site thereof with a nucleophile to yield
15 a diol; said nucleophile being selected from the group consisting of an aryl alcohol, and arylthioic acid, and a heterocyclic dithioic acid;

(e) esterifying said diol with an alpha-amino acid optionally bearing an N-protecting
20 group to yield a diester; said N-protecting group being selected from the group consisting of benzyloxycarbonyl and t-butoxycarbonyl; and

(f) treating said diester with a mixture of water and said acid of step (a) to yield
25 said aspartyl protease inhibitor.

Another method for synthesizing aspartyl protease inhibitors according to this invention comprises the steps of:

(a) treating mannitol with a carbonyl
30 compound in the presence of an acid to yield a ketal derivative; said carbonyl compound being selected from the group consisting of acetone, cyclopentanone, cyclohexanone, and benzaldehyde; and said acid being selected from the group consisting of sulfuric,

- 50 -

hydrochloric, acetic, trifluoroacetic, and mixtures thereof;

(b) esterifying said ketal derivative at each primary hydroxyl site thereof with an esterifying agent to yield a diol; said esterifying agent being selected from the group consisting of a carboxylic acid, a carboxylic acid halide, and a carboxylic acid anhydride;

(c) esterifying said diol with an alpha-amino acid optionally bearing an N-protecting group to yield a diester; said N-protecting group being selected from the group consisting of benzyloxycarbonyl and t-butoxycarbonyl; and

(d) treating said diester with a mixture of water and said acid of step (a) to yield said aspartyl protease inhibitor.

EXAMPLES

In order that this invention be more fully understood, the following examples are set forth. These examples are for the purpose of illustration only and are not to be construed as limiting the scope of the invention in any way.

Materials and Methods

The Al_2O_3 employed for epoxide ring-opening reactions with heteroatom nucleophiles was preferably Brockman Super I, Woelm 200 neutral (from ICN flow). L-mannitol was prepared by the reduction of L-mannose which was purchased from Sigma. Commercial L-amino acid derivatives were employed where relevant.

Analytical thin layer chromatography (TLC) was carried out with 0.25 mm silica gel E. Merck 60 F₂₅₄ plates and eluted with the indicated solvent systems.

- 51 -

Preparative chromatography was performed either by flash chromatography, using Silica Gel 60 (EM Science) with the indicated solvent systems and a positive N₂ pressure for elutions, or by thick layer chromatography, again employing E. Merck 60 F₂₅₄ plates of 0.5, 1.0, or 2.0 mm thickness. Detection of the compounds was carried out by exposing eluted plates (analytical or preparative) to UV light and treating analytical plates with a 20% solution of phosphomolybdic acid in EtOH, followed by heating, unless otherwise indicated.

All analytical HPLC was carried out with a Waters Delta Pak, 5 μ M silica, C-18 reverse-phase column with dimensions of 3.90 mm ID x 15 cm, using a flow rate of 1.5 ml/min., with the following mobile phase and gradient profile:

Mobile phase: A=0.1% trifluoroacetic acid in H₂O;
B=0.1% trifluoroacetic acid in CH₃CN

Gradient: T=0 min., A (95%), B (5%);
T=20 min., A (0%), B (100%);
T=22.5 min., A (0%), B (100%)

Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AMX 500 equipped with a reverse or QNP probe. Samples were dissolved in deuteriochloroform or deuteriomethanol for data acquisition, using the respective internal protic solvent frequencies as standards. Chemical shifts (in parts per million) and multiplicities (denoted as s for singlet, d for doublet, dd for doublet of doublets, t for triplet, q for quartet, m for multiplet, and br for broad) are provided herein, and the number of hydrogens listed for all C₂-symmetric compounds is half of that present in the molecule.

- 52 -

The following compounds were prepared from L-mannitol using the procedures described by Le Merrer et al., Heterocycles, 25, pp. 541-48, (1987), wherein D-mannitol was utilized for compounds 1B, 1C, 1D, and 5A.

EXAMPLE 1

A. L-mannitol (Compound 1A).

To a commercial sample of L-mannose as a mixture of anomers (27.2 g, 0.15 mol) in MeOH (700 ml) was placed sodium borohydride (12.6 g, 0.33 mol) in portions at 0°C, and the reaction was acidified to pH-1 by the dropwise addition of concentrated HCl at 0°C, then solid K₂CO₃ (excess) was added and the solution was vacuum filtered using MeOH for quantitative transfer. Concentration of the filtrate afforded a white solid that was azeotroped with benzene (2 x 200 mL). This was followed by crystallization of the solid from alcohol (1.8 L), which was effected by heating the solution at reflux and filtering it immediately. L-mannitol crystallized rapidly as white needles to provide 17.3 g of the product, compound 1A (63% yld). TLC: R_f (9% NH₄OH/18% MeOH/CH₂Cl₂) = 0.6 (visualized with ninhydrin); NMR (CD₃OD): 3.86 (dd, 1H, ABX), 3.68 (dd, 1H, ABX), 3.82 (s, 1H), 3.74 (m, 1H).

B. 1,2:3,4:5,6-tris-O-isopropylidene-L-mannitol (Compound 1B).

L-Mannitol (15.5 g, 0.09 mol) was diluted with acetone (193 ml) and sulfuric acid (1.55 ml), and the reaction (which remained heterogeneous throughout) stirred at room temperature for 12 hours. It was neutralized with 30% NH₄OH (5.6 ml) and solid K₂CO₃ (9.8 g), with continued stirring for 2 hours, followed by vacuum filtration. Concentration of the filtrate

- 53 -

afforded a white solid which crystallized from wet alcohol by adding enough EtOH and warming until homogeneous, then adding H₂O dropwise with swirling until the solution became slightly cloudy. After
5 crystallization, compound 1B was recovered by vacuum filtration as white, crystalline needles (21 g). More compound could be recovered from the mother liquor, if desired. NMR (CDCl₃): 4.16 (m, 1H), 4.05 (dd, 1H, ABX), 3.95 (dd, 1H, ABX), 3.92 (dd, 1H), 1.40 (s, 3H),
10 1.35 (s, 3H), 1.32 (s, 3H).

C. 3,4-O-isopropylidene-L-mannitol (Compound 1C).

To a mixture of distilled H₂O (120 mL) and glacial acetic acid (280 mL) was added compound 1B (19.8 g, 0.66 mol) at 40°C, and the reaction was
15 maintained at this temperature for 2.5 hours. The aqueous acetic acid was removed immediately in vacuo, maintaining the bath temperature between 40-50°C. The resulting white solid was diluted with acetone (150 ml), and the undissolved white precipitate (L-mannitol)
20 was removed by vacuum filtration. Concentration of the filtrate provided a clear, viscous oil which soon crystallized in vacuo. Recrystallization from benzene afforded compound 1C as a white, crystalline product (8.3 g). NMR (CD₃OD): 3.98 (dd, 1H), 3.81 (dd, 1H, ABX), 3.66 (dd, 1H, ABX), 3.69 (m, 1H), 1.41 (s, 3H).
25

D. 1,6-di-O-benzoyl-3,4-O-isopropylidene-L-mannitol (Compound 1D).

To a solution of compound 1C (45 mg, 0.20 mmol) and pyridine (0.83 mL, 10.26 mmol) in CH₂Cl₂ (1
30 ml) was added benzoyl chloride (47 µl, 0.41 mmol) at -78°C, the system under a N₂ atmosphere. After stirring for 2 hours at this temperature, the reaction was warmed to 20°C and poured into cold 6N HCl (2 ml), then

- 54 -

extracted with CH_2Cl_2 . The organic layer was washed with saturated NaHCO_3 and dried over MgSO_4 . After filtration and concentration, the resulting residue was flash chromatographed (9% $\text{Et}_2\text{O}/\text{CH}_2\text{Cl}_2$) to yield compound 1D (25 mg). NMR (CDCl_3): 8.04 (d, 2H), 7.56 (t, 1H), 7.41 (t, 2H), 4.69 (dd, 1H, ABX), 4.42 (dd, 1H, ABX), 4.05 (s, 1H), 3.98 (m, 1H), 1.39 (s, 3H).

E. 1,6-di-O-benzoyl-2,5-di-O-(N-carbobenzyloxyvalyl)-3,4-O-isopropylidene-L-mannitol (Compound 1E).

Compound 1D (25 mg, 0.06 mmol) was stirred in CH_2Cl_2 (2 ml) in the presence of carbobenzyloxyvaline (CbzVal-OH; 73 mg, 0.29 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC; 56 mg, 0.29 mmol), and dimethylaminopyridine (DMAP; catalytic), for 12 hours at ambient temperature. The reaction mixture was then diluted with CH_2Cl_2 , and the organic layer was washed with saturated NaHCO_3 and dried over MgSO_4 . After filtration and concentration, the resulting residue was flash chromatographed (10% $\text{Et}_2\text{O}/\text{CH}_2\text{Cl}_2$) to afford compound 1E as a colorless oil (20 mg). NMR (CDCl_3): 7.99 (d, 2H), 7.51 (t, 1H), 7.21-7.40 (m, 1H), 5.31 (m, 2H), 5.08 (d, 1H, AB), 4.95 (d, 1H, AB), 4.77 (d, 1H, ABX), 4.41 (dd, 1H, ABX), 4.35 (m, 1H), 4.25 (d, 1H), 2.17 (m, 1H), 1.46 (s, 3H), 0.93 (d, 3H), 0.82 (d, 3H).

F. 1,6-di-O-benzoyl-2,5-di-O-(N-carbobenzyloxyvalyl)-L-mannitol (Compound 1).

Deprotection of the acetonide was effected by stirring compound 1E (5 mg) with 90% aqueous trifluoroacetic acid (TFA; 1 ml), at 0°C for 70 minutes, then removing the solvent in vacuo. The resulting residue was purified by flash chromatography using 25% $\text{Et}_2\text{O}/\text{CH}_2\text{Cl}_2$ as eluent, which afforded compound

- 55 -

1 (1.4 mg). TLC: R_f (25% Et₂O/CH₂Cl₂) = 0.3; analytical HPLC: R_t = 19.1 minutes.

EXAMPLE 2

5 A. 3-(t-butyldimethylsiloxy)-benzoic acid (Compound 2A).

 A solution of 3-hydroxybenzoic acid (219 mg, 1.59 mmol), t-butyldimethylsilyl chloride (717 mg, 4.76 mmol), and imidazole (324 mg, 4.76 mmol) in DMF (3 ml) was stirred for 12 hours at room temperature. The
10 reaction mixture was diluted with EtOAc and washed with saturated NH₄Cl. The organic layer was washed with H₂O (two times) and dried over MgSO₄. Filtration and concentration gave a colorless liquid (831.4 mg). The resulting colorless liquid product (565 mg) was
15 dissolved in 60%/20%/20%, acetic acid/H₂O/THF (5 ml), and the reaction proceeded at room temperature for 90 minutes. Removal of the solvent in vacuo afforded compound 2A (125 mg) as a solid. NMR (CDCl₃): 7.70 (d, 1H), 7.55 (br s, 1H), 7.32 (t, 1H), 7.07 (d, 1H), 0.99
20 (s, 9H), 0.21 (s, 6H).

B. 1,6-di-O-[3-(t-butyldimethylsiloxy)-benzoyl]-3,4-O-isopropylidene L-mannitol (Compound 2B).

 To a solution of 3-(t-butyldimethylsiloxy)-benzoic acid (compound 2A) (125 mg, 0.50 mmol) and
25 compound 1C (55 mg, 0.25 mmol) in CH₂Cl₂ (3 ml) was placed EDC (95 mg, 0.50 mmol) and DMAP (catalytic), and the resulting mixture was stirred for 12 hours. Removal of the solvent in vacuo gave a residue which was flash chromatographed with 9% Et₂O/CH₂Cl₂, affording
30 compound 2B as a colorless residue (78.4 mg) which was used in the next reaction without purification.

- 56 -

C. 1,6-di-O-[3-(t-butyldimethylsiloxy)-benzoyl]-2,5-di-O-(N-carbobenzyloxyvalyl)-3,4-O-isopropylidene-L-mannitol (Compound 2C).

Following the method of Example 1E, compound 2B (28.4 mg), CbzVal-OH (52 mg, 0.21 mmol), EDC (39 mg, 0.21 mmol) and DMAP (catalytic in 2 ml CH₂Cl₂) were allowed to react. Removal of the solvent in vacuo provided a residue which was flash chromatographed with 9% Et₂O/CH₂Cl₂, affording compound 2C as a colorless oil (51.5 mg). TLC: R_f (9% Et₂O/CH₂Cl₂) = 0.72.

D. 1,6-di-O-(3-hydroxy)benzoyl-2,5-di-O-(N-carbobenzyloxyvalyl)-L-mannitol (Compound 2).

Following the method of Example 1F, compound 2C (44.1 mg, 0.05 mmol) was converted to 22.2 mg of compound 2 (concomitant silyl and acetonide removal occurred under the reaction conditions) after preparative TLC (0.25 mm) using 5% MeOH/CH₂Cl₂ as eluent. TLC: R_f (5% MeOH/CH₂Cl₂) = 0.59; analytical HPLC: R_t = 17.0 minutes.

EXAMPLE 3

A. 1,6-di-O-nicotinoyl-3,4-O-isopropylidene-L-mannitol (Compound 3A).

Nicotinoyl chloride hydrochloride (324.1 mg, 1.82 mmol) was added in one portion to a -78°C solution of compound 1C (202.4 mg, 0.91 mmol) in pyridine (3.6 ml) and CH₂Cl₂ (3.6 ml). The reaction was allowed to stir for 5 hours, while slowly warming to room temperature. The reaction was poured onto CH₂Cl₂ (40 ml) and washed with H₂O (three times) and saturated aqueous NaCl, then dried over Na₂SO₄ and concentrated in vacuo. Flash chromatography using 15% isopropanol/CH₂Cl₂ as eluent gave compound 3A (209 mg). NMR (CDCl₃): 9.20 (s, 1H), 8.72 (d, 1H), 8.29 (d, 1H), 7.38

- 57 -

(m, 1H), 5.23 (br s, 1H), 4.74 (d, 1H), 4.47 (m, 1H), 4.03 (dd, 2H), 1.42 (s, 3H).

B. 1,6-di-O-nicotinoyl-2,5-di-O-(N-carbobenzyloxyvalyl)-3,4-O-isopropylidene-L-mannitol (Compound 3B).

5 CbzVal-OH (815.7 mg, 3.25 mmol) and compound
3A (200 mg, 0.46 mmol) in CH₂Cl₂ (5 ml) was treated with
EDC (620.7 mg, 3.23 mmol) and DMAP (11.3 mg, 0.09
mmol), and the resulting mixture was stirred for 48
10 hours. The reaction was diluted with CH₂Cl₂ (40 ml) and
washed with H₂O (two times) and saturated aqueous NaCl,
then dried over Na₂SO₄ and concentrated in vacuo. Flash
chromatography with 3.5% isopropanol/CH₂Cl₂ as eluent
provided compound 3B (340 mg) as a white foam. NMR
15 (CDCl₃): 9.20 (s, 1H), 8.74 (d, 1H), 8.23 (d, 1H),
7.20-7.40 (m, 7H), 5.32 (m, 2H), 5.02 (dd, 2H), 4.80
(d, 1H), 4.50 (m, 1H), 4.31 (m, 1H), 4.23 (d, 1H), 2.20
(m, 1H), 1.45 (s, 3H), 0.93 (d, 3H), 0.84 (d, 3H).

C. 1,6-di-O-nicotinoyl-2,5-di-O-(N-carbobenzyloxyvalyl)-L-mannitol (Compound 3).

20 Compound 3B (320 mg, 0.36 mmol) was stirred
with 90% aqueous trifluoroacetic acid (1.1 ml) and
CH₂Cl₂ (2 ml) for 18 hours at room temperature, followed
by concentration in vacuo. Preparative HPLC
25 (reverse-phase C-18 column, 30 mm x 30 cm, 100
angstrom, mobile phase: 70% to 20% A/B over a 60 minute
gradient elution) gave 59.6 mg of compound 3 and 158.8
mg of recovered compound 3B. TLC: R_f (7%
isopropanol/CH₂Cl₂) = 0.42; analytical HPLC: R_t = 14.9
30 minutes.

- 58 -

EXAMPLE 4

A. 1,6-di-O-[4-(1,2,3-thiadiazolyl)-3,4-O-isopropylidene-L-mannitol (Compound 4A)].

Following the method of Example 1D, compound
5 1C (49.0 mg, 0.22 mmol) was treated with 1,2,3-
thiadiazole-4-carbonyl chloride (0.57 mmol).
Preparative TLC (0.5 mm) using 5% MeOH/CH₂Cl₂ as eluent
gave 20.9 mg of compound 4A as a yellow solid. NMR
(CDCl₃/DMSO-d₆): 9.8 (s, 1H), 5.63 (br s, 1H), 4.67 (d,
10 1H), 4.40 (dd, 1H), 4.11 (d, 1H), 4.01 (br s, 1H), 1.41
(s, 3H).

B. 1,6-di-O-[4-(1,2,3-thiadiazolyl)-2,5-di-O-(N-carbobenzyloxyvalyl)-3,4-O-isopropylidene-L-mannitol (Compound 4B)].

15 CbzVal-OH (72.3 mg, 0.29 mmol) and compound
4A (18 mg, 0.04 mmol) in DMF (1.2 ml) were treated with
EDC (53.3 mg, 0.28 mmol) and DMAP (2.4 mg, 0.02 mmol),
and the resulting mixture was stirred for 24 hours.
The reaction was diluted with EtOAc and washed with
20 cold 3N HCl (two times), cold aqueous 5% NaHCO₃ (two
times), and saturated aqueous NaCl, then dried over
Na₂SO₄ and concentrated in vacuo. Preparative TLC (0.5
mm) with 4% MeOH/CH₂Cl₂ as eluent gave 27.4 mg of
compound 4B as a white foam. NMR (CDCl₃): 9.28 (s,
25 1H), 7.20-7.40 (m, 5H), 5.25-5.35 (m, 2H), 4.98-5.15
(m, 2H), 4.90 (d, 1H), 4.51 (dd, 2H), 4.30 (m, 1H),
2.20 (br s, 1H), 1.45 (s, 3H), 0.97 (d, 3H), 0.85 (s,
3H).

C. 1,6-di-O-[4-1,2,3-thiadiazolyl-2,5-di-O-(N-carbobenzyloxyvalyl)-L-mannitol (Compound 4)].

30 Following the method of Example 3C, compound
4B (27.2 mg, 0.03 mmol) was converted to 5.7 mg of
compound 4 after preparative TLC (0.5 mm) using 5%

- 59 -

isopropanol/CH₂Cl₂ as eluent. TLC: R_f (5% isopropanol/CH₂Cl₂) = 0.50; analytical HPLC: R_t = 16.9 minutes.

EXAMPLE 5

5 A. 1,2,5,6-dianhydro-3,4-O-isopropylidene L-mannitol
 (Compound 5A).

To compound 1C (5.2 g, 0.02 mol) in pyridine (75 ml, 0.93 mol) was added p-toluenesulfonyl chloride (9.2 g, 0.05 mol) in portions at 0°C. After stirring
10 for 3 hours at this temperature, the reaction mixture was poured into a cold 6N HCl (160 ml)/Et₂O (75 ml) mixture, and the organic layer was separated and washed with a 3% NaHCO₃ solution (100 ml), then was dried over MgSO₄. Filtration and concentration afforded the
15 desired bis-tosylate, which was used immediately in the next reaction without purification.

Anhydrous K₂CO₃ (16.2 g, 0.12 mol) was added to a solution of the preceding isolate in anhydrous MeOH (150 ml), and the reaction proceeded at room
20 temperature for 3 hours. The reaction mixture was then diluted with CH₂Cl₂ and washed successively with H₂O and saturated NH₄Cl, followed by drying over MgSO₄. Filtration and concentration gave the crude bis-epoxide as a pale yellow liquid, which was distilled under
25 reduced pressure (-0.5 mm Hg) to provide compound 5A (2.1 g) as a colorless oil (b.p.: 75-82°C). NMR (CDCl₃): 3.80 (dd, 1H), 3.08 (dd, 1H), 2.81 (t, 1H), 2.68 (dd, 1H), 1.40 (s, 3H).

30 B. 1,6-di-O-benzyl-3,4-O-isopropylidene-L-mannitol
 (Compound 5B).

Benzyl alcohol (104 μl, 1.00 mmol) was added to a slurry of Al₂O₃ (1.35 g) in Et₂O (3 ml), and after

- 60 -

stirring for 5 minutes, a solution of the bis-epoxide compound 5A (34.2 mg, 0.18 mmol) in Et₂O (2 ml) was added. The reaction proceeded at room temperature for 12 hours, then MeOH (20 ml) was added to the reaction mixture and the solution was allowed to stand for 4 hours. After vacuum filtration of the solution over a Celite® pad, concentration gave a residue which was purified by thick layer chromatography (0.5 mm) using 9% Et₂O/CH₂Cl₂ as eluent to provide compound 5B (3.0 mg).

C. 1,6-di-O-benzyl-2,5-di-O-(N-carbobenzyloxyvalyl)-3,4-O-isopropylidene-L-mannitol (Compound 5C).

Compound 5B (3.0 mg, 0.01 mmol) was stirred in CH₂Cl₂ (2 ml) in the presence of CbzVal-OH (10 mg, 0.04 mmol), EDC (8 mg, 0.04 mmol), and DMAP (catalytic) for 12 hours. Concentration of the reaction mixture, followed by flash chromatography of the residue (9% Et₂O/CH₂Cl₂), gave the coupled product, compound 5C (1.9 mg).

D. 1,6-di-O-benzyl-2,5-di-O-(N-carbobenzyloxyvalyl)-L-mannitol (Compound 5).

Acetonide removal was effected by stirring compound 5C (1.9 mg, 2.19 μmol) with 90% aqueous trifluoroacetic acid (1.5 ml) at 0°C for 75 minutes, then removing the solvent in vacuo. The resulting residue was purified by thick layer chromatography (0.5 mm) using 17% Et₂O/CH₂Cl₂ as eluent, which afforded compound 5 (1.6 mg) as a white solid. TLC: R_f (50% hexane/EtOAc) = 0.3; NMR (CDCl₃): 7.18-7.40 (m, 10H), 5.20 (br d, 1H), 4.95-5.15 (m, 3H), 4.49 (m, 2H), 4.30 (dd, 1H), 3.85 (t, 1H), 3.75 (br s, 2H), 2.98 (d, 1H), 2.15 (m, 1H), 0.93 (d, 3H), 0.81 (d, 3H).

- 61 -

EXAMPLE 6A. 1,6-di-S-benzoyl-3,4-O-isopropylidene-L-mannitol (Compound 6A).

To a solution of thiobenzoic acid (136 μ l, 1.15 mmol) and Al_2O_3 (1.51 g) in Et_2O (2 ml) was placed, after 5 minutes, a solution of the epoxide compound 5A (42.9 mg, 0.23 mmol) in THF (1 ml), and the reaction stirred at room temperature for 12 hours. MeOH (20 ml) was added, and the reaction mixture stirred for 3 hours, then was vacuum filtered. After removal of the solvent in vacuo, the residue was dissolved in EtOAc and washed with saturated NaHCO_3 , then dried over MgSO_4 . Filtration and concentration gave compound 6A as a purple oil (77.1 mg), which was used in the next reaction without purification. TLC: R_f (50% hexane/EtOAc) = 0.63.

B. 1,6-di-S-benzoyl-2,5-di-O-(N-carbobenzyloxyvalyl)-3,4-O-isopropylidene-L-mannitol (Compound 6B).

Compound 6A (77.1 mg) was stirred in CH_2Cl_2 (2 ml) in the presence of CbzVal-OH (293 mg, 1.17 mmol), EDC (224 mg, 1.17 mmol) and DMAP (4 mg, 0.03 mmol), and the resulting mixture was stirred for 12 hours. Removal of the solvent in vacuo and purification of the residue by preparative TLC (3X 1.0 mm) with 25% EtOAc/hexane as eluent gave compound 6B (124 mg). TLC: R_f (50% hexane/EtOAc) = 0.82.

C. 1,6-di-S-benzoyl-2,5-di-O-(N-carbobenzyloxyvalyl)-L-mannitol (Compound 6).

Following the method of Example 1F, compound 6B (23.4 mg, 0.03 mmol) was converted to 6.4 mg of compound 6 after preparative TLC (0.5 mm) using 25% $\text{Et}_2\text{O}/\text{CH}_2\text{Cl}_2$. TLC: R_f (25% $\text{Et}_2\text{O}/\text{CH}_2\text{Cl}_2$) = 0.43; analytical HPLC: R_t = 19.8 minutes.

- 62 -

EXAMPLE 7A. 1,6-di-S-[4-morpholinothiocarbonyl]-3,4-O-isopropylidene-L-mannitol (Compound 7A).

To a solution of 4-morpholinecarbodithioic
5 acid with morpholine as a 1:1 complex (239 mg, 0.95
mmol) in DMF (1.5 ml) was added a solution of the
bis-epoxide compound 5A (59.2 mg, 0.32 mmol) in Et₂O
(0.5 ml), and the reaction proceeded at room
temperature for 12 hours. After dilution with EtOAc,
10 the reaction mixture was washed with H₂O (three times),
and the combined aqueous layers were extracted with
EtOAc, which in turn was washed with H₂O (two times).
The combined organic layers were dried over MgSO₄.
Filtration and concentration afforded a residue which
15 was purified by thick layer chromatography (1.0 mm)
using EtOAc as eluent to yield diol compound 7A (78 mg)
as a white solid. TLC: R_f (EtOAc) = 0.63.

B. 1,6-di-S-(4-morpholinothiocarbonyl)-2,5-di-O-(N-
20 carbobenzyloxyvalyl)-3,4-O-isopropylidene-L-mannitol
(Compound 7B).

Compound 7A (38.9 mg, 0.08 mmol) was stirred
in CH₂Cl₂ (1 ml) in the presence of CbzVal-OH (133 mg,
0.53 mmol), EDC (102 mg, 0.53 mmol), and DMAP
(catalytic) for 48 hours. The reaction mixture was
25 then diluted with CH₂Cl₂ and washed with saturated
NaHCO₃, and the organic layer was dried over MgSO₄.
Filtration and concentration gave a residue which was
purified by thick layer chromatography (1.0 mm) using
50% hexane/EtOAc as eluent to provide the coupled
30 product, compound 7B (46 mg). TLC: R_f (50%
hexane/EtOAc) = 0.34.

- 63 -

C. 1,6-di-S-(4-morpholinothiocarbonyl)-2,5-di-O-(N-carbobenzyloxyvalyl)-L-mannitol (Compound 7).

The acetonide was removed by stirring compound 7B (12.6 mg, 0.01 mmol) with 90% aqueous trifluoroacetic acid (1 ml) at 0°C, and allowing the reaction mixture to reach ambient temperature over a 2.5 hour period. After removal of the solvent in vacuo, the resulting residue was purified by thick layer chromatography (0.5 mm) using 5% MeOH/CH₂Cl₂ as eluent, which afforded compound 7 (7.4 mg) as a colorless oil. TLC: R_f (5% MeOH/CH₂Cl₂) = 0.51; analytical HPLC: R_t = 18.1 minutes.

EXAMPLE 8

A. 1,6-di-S-(N,N-diethylthiocarbonyl)-3,4-O-isopropylidene L-mannitol (Compound 8A).

Following the method of Example 7A, the ammonium salt of diethyldithiocarbamic acid (133 mg, 0.80 mmol) and the epoxide compound 5A (49.8 mg, 0.27 mmol) were reacted to provide compound 8A (88 mg), which was used in the next reaction without purification.

B. 1,6-di-S-(N,N-diethylthiocarbonyl)-2,5-di-O-(N-carbobenzyloxyvalyl)-3,4-O-isopropylidene L-mannitol (Compound 8B).

Compound 8A (88 mg) was stirred in CH₂Cl₂ (3 ml) in the presence of CbzVal-OH (319 mg, 1.27 mmol), EDC (244 mg, 1.27 mmol), and DMAP (catalytic) for 12 hours. The reaction mixture was then diluted with CH₂Cl₂ and washed with saturated NaHCO₃, and the organic layer was dried over MgSO₄. Filtration and concentration gave a residue which was purified by flash chromatography with 33% EtOAc/hexane as eluent to

- 64 -

afford the coupled product, compound 8B (180 mg). TLC: R_f (33% EtOAc/hexane) = 0.25.

C. 1,6-di-S-(N,N-diethylthiocarbonyl)-2,5-di-O-(N-carbobenzyloxyvalyl-L-mannitol (Compound 8)).

5 Following the method of Example 1F, compound 8B (28.7 mg, 0.03 mmol) was converted to 12.1 mg of compound 8 after preparative TLC (2 x 0.25 mm) using 5% MeOH/CH₂Cl₂ as eluent. TLC: R_f (5% MeOH/CH₂Cl₂) = 0.6; analytical HPLC: R_t = 19.8 minutes.

10

EXAMPLE 9

As recorded in Table II, we measured the inhibition constants of various compounds of this invention against HIV-1 protease, using the method described essentially by M.W. Pennington et al.,
15 Peptides 1990, Gimet, E. and D. Andrew, Eds., Escom; Leiden, Netherlands (1990).

TABLE II

	<u>Compound</u>	<u>K_i (nM)</u>
	1	35
20	2	130
	3	100
	4	400
	5	60
	6	400
25	7	20
	8	250

As demonstrated in Table II, all of the compounds tested displayed inhibitory and anti-viral activity.

30

While we have described a number of embodiments of this invention, it is apparent that our basic constructions may be altered to provide other embodiments which utilize the products and processes of this invention. Therefore, it will be appreciated that

- 65 -

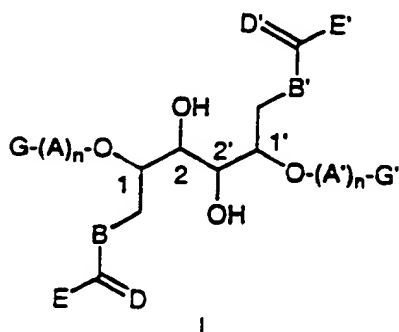
the scope of this invention is to be defined by the appended claims, rather than by the specific embodiments which have been presented by way of example.

- 66 -

CLAIMS

We claim:

1. A compound of formula I:



wherein:

each n is independently selected from the group consisting of 0, 1 and 2;

each A and A' is independently selected from the group consisting of a naturally occurring alpha-amino acid and an unnatural alpha-amino acid, wherein the amino group of each A or A' is bonded to G or G' or to the carboxy group of the adjacent residue A or A' , whichever is appropriate, and the carboxy group of A or A' is bonded to the amino group of the adjacent residue A or A' or to the oxygen of the structure, whichever is appropriate;

each G and G' is independently covalently attached to the amino group of the adjacent residue A or A' or to the oxygen of the structure (if the adjacent $n = 0$) and is selected from the group consisting of trityl; hydrogen; C_1 - C_6 alkyl; R_3 -CO-; $R_5(R_6R_7C)_m$ CO-; $R_5(R_6R_7C)_m$ W-; R_8 -W-; $R_5(R_6R_7C)_m$ -P(O)(OR₉)-; R_8 -P(O)(OR₉)-; and phthaloyl, which may be optionally substituted with one or more substituents R_4 ; wherein $m = 1-3$;

each W is independently selected from the group consisting of -OCO- and -SO₂-, with the proviso

- 67 -

that W is not $-SO_2-$ when W is attached to the oxygen of the structure;

each R_3 is independently selected from the group consisting of hydrogen; C_1-C_6 alkyl; C_2-C_6 alkenyl; phenyl; and naphthyl; said C_1-C_6 alkyl and C_2-C_6 alkenyl being optionally substituted with one or more substituents selected from the group consisting of hydroxyl, chlorine, and fluorine; and said phenyl and naphthyl being optionally substituted with one or more substituents R_4 ;

each R_4 is independently selected from the group consisting of C_1-C_4 alkyl; C_2-C_4 alkenyl; halogen; hydroxyl; nitro; C_1-C_3 alkoxy; and $-CO-N(R_{10})(R_{10})$;

each R_5 , R_6 and R_7 is independently selected from the group consisting of hydroxyl; hydrogen; chlorine; fluorine; C_1-C_3 alkoxy; a 5-7 membered heterocycle; C_1-C_3 alkyl; phenyl; and naphthyl; said C_1-C_3 alkyl being optionally substituted with one or more substituents selected from the group consisting of chlorine, fluorine, and hydroxyl; and said phenyl and naphthyl being optionally substituted with one or more substituents R_4 ; or R_5 , R_6 and R_7 of a particular G or G' taken individually, or together in any combination, may be optionally joined to form a monocyclic, bicyclic, or tricyclic ring system, each ring of which is C_3-C_6 cycloalkyl; with the proviso that R_5 , R_6 , or R_7 may not be chlorine, fluorine, or hydroxyl if it is present on the carbon adjacent to W;

each R_8 is independently selected from the group consisting of a 5-7 membered heterocycle; phenyl; and naphthyl; said phenyl and naphthyl being optionally substituted with one or more substituents R_4 ;

each R_9 is independently selected from the group consisting of C_1-C_4 alkyl and phenyl;

- 68 -

each R_{10} is independently selected from the group consisting of hydrogen; C_1-C_4 alkyl; and a 5-7 membered heterocycle;

each B and B' is independently selected from the group consisting of oxygen and sulfur;

each D and D' is independently selected from the group consisting of H_2 , oxygen and sulfur;

each E and E' is independently selected from the group consisting of Ar and $N(R_{11}R_{12})$, with the proviso that when both $n = 0$, E and E' are not 3-pyridyl or diethylamino;

each Ar is independently selected from the group consisting of phenyl and a 5-7 membered heterocycle; said phenyl and heterocycle being optionally substituted with one or more substituents R_4 ; and

each R_{11} and R_{12} is independently selected from the group consisting of C_1-C_6 alkyl and C_2-C_6 alkenyl.

2. The compound according to claim 1, wherein:

each n is independently selected from the group consisting of 0, 1 and 2;

each A and A' is independently selected from the group consisting of a naturally occurring alpha-amino acid and an unnatural alpha-amino acid, wherein the amino group of each A or A' is bonded to G or G' or to the carboxy group of the adjacent residue A or A', whichever is appropriate, and the carboxy group of A or A' is bonded to the amino group of the adjacent residue A or A' or to the oxygen of the structure, whichever is appropriate;

each G and G' is independently covalently attached to the amino group of the adjacent residue A

- 69 -

or A' or to the oxygen of the structure (if the adjacent $n = 0$), and is selected from the group consisting of hydrogen; C_1-C_6 alkyl; R_3CO- ; $R_5(R_6R_7C)_mCO-$; $R_5(R_6R_7C)_mW-$; and phthaloyl, which may be optionally substituted with one or more substituents R_4 ; wherein $m = 1-3$;

each W is independently selected from the group consisting of $-OCO-$ and $-SO_2-$, with the proviso that W is not $-SO_2-$ when W is attached to the oxygen of the structure;

each R_3 is independently selected from the group consisting of C_1-C_6 alkyl; phenyl; and naphthyl; said C_1-C_6 alkyl being optionally substituted with one or more substituents selected from the group consisting of hydroxyl, chlorine, and fluorine; and said phenyl and naphthyl being optionally substituted with one or more substituents R_4 ;

each R_4 is independently selected from the group consisting of C_1-C_4 alkyl; halogen; hydroxyl; nitro; and C_1-C_3 alkoxy;

each R_5 , R_6 , and R_7 is independently selected from the group consisting of hydrogen; chlorine; fluorine; C_1-C_3 alkyl; C_1-C_3 alkoxy; a 5-7 membered heterocycle; phenyl; and naphthyl; said C_1-C_3 alkyl being optionally substituted with one or more substituents selected from the group consisting of chlorine, fluorine, and hydroxyl; and said phenyl and naphthyl being optionally substituted with one or more substituents R_4 ; or R_5 , R_6 , and R_7 of a particular G or G' taken individually, or together in any combination, may be optionally joined to form a monocyclic, bicyclic, or tricyclic ring system, each ring of which is C_3-C_6 cycloalkyl; with the proviso that R_5 , R_6 , or R_7

- 70 -

may not be chlorine, fluorine, or hydroxyl if it is present on the carbon adjacent to W;

each B and B' is independently selected from the group consisting of oxygen and sulfur;

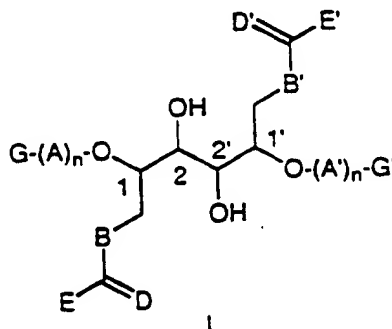
each D and D' is independently selected from the group consisting of H₂; oxygen and sulfur;

each E and E' is independently selected from the group consisting of Ar and N(R₁₁R₁₂), with the proviso that each E and E' is not 3-pyridyl or diethylamino when both n = 0;

each Ar is independently selected from the group consisting of phenyl and a 5-7 membered heterocycle; wherein said phenyl and 5-7 membered heterocycle may be optionally substituted with one or more substituents R₄; and

each R₁₁ and R₁₂ is independently C₁-C₆ alkyl.

3. A compound of formula I:



wherein:

each n is independently selected from the group consisting of 0, 1 and 2;

each A and A' is independently selected from the group consisting of a naturally occurring alpha-amino acid and an unnatural alpha-amino acid, wherein the amino group of each A or A' is bonded to G or G' or to the carboxy group of the adjacent residue A or A', whichever is appropriate, and the carboxy group of A or

- 71 -

A' is bonded to the amino group of the adjacent residue A or A' or to the oxygen of the structure, whichever is appropriate;

each G and G' is independently covalently attached to the amino group of the adjacent A or A' or to the oxygen of the structure (if the adjacent n = 0), and is selected from the group consisting of trityl; C₁-C₆ alkyl; R₃-CO-; R₅(R₆R₇C)_mCO-; R₅(R₆R₇C)_mW-; R₈-W-; R₅(R₆R₇C)_m-P(O)(OR₉)-; R₈-P(O)(OR₉)-; and phthaloyl, which may be optionally substituted with one or more substituents R₄; wherein m = 1-3;

each W is independently selected from the group consisting of -OCO- and -SO₂-, with the proviso that W is not -SO₂- when W is attached to the oxygen of the structure;

each R₃ is selected from the group consisting of hydrogen; C₁-C₆ alkyl; C₂-C₆ alkenyl; phenyl; and naphthyl; said C₁-C₆ alkyl and C₂-C₆ alkenyl being optionally substituted with one or more substituents selected from the group consisting of hydroxyl, chlorine, and fluorine; and said phenyl and naphthyl being optionally substituted with one or more substituents R₄;

each R₄ is independently selected from the group consisting of C₁-C₄ alkyl; C₂-C₄ alkenyl; halogen; hydroxyl; nitro; C₁-C₃ alkoxy; and -CO-N(R₁₀)(R₁₀);

each R₅, R₆ and R₇ is independently selected from the group consisting of hydroxyl; hydrogen; chlorine; fluorine; C₁-C₃ alkoxy; a 5-7 membered heterocycle; C₁-C₃ alkyl; phenyl; and naphthyl; said C₁-C₃ alkyl being optionally substituted with one or more substituents selected from the group consisting of chlorine, fluorine and hydroxyl; and said phenyl and naphthyl being optionally substituted with one or more

- 72 -

substituents R_4 ; or R_5 , R_6 and R_7 of a particular G or G' taken individually, or together in any combination, may be optionally joined to form a monocyclic, bicyclic, or tricyclic ring system, each ring of which is C_3 - C_6 cycloalkyl; with the proviso that R_5 , R_6 , and R_7 may not be chlorine, fluorine, or hydroxyl if it is present on the carbon adjacent to W;

each R_8 is independently selected from the group consisting of a 5-7 membered heterocycle; phenyl; and naphthyl; said phenyl and naphthyl being optionally substituted with one or more substituents R_4 ;

each R_9 is independently selected from the group consisting of C_1 - C_4 alkyl and phenyl;

each R_{10} is independently selected from the group consisting of hydrogen; C_1 - C_4 alkyl; and a 5-7 membered heterocycle;

each B and B' is independently selected from the group consisting of oxygen and sulfur;

each D and D' is independently selected from the group consisting of H_2 , oxygen and sulfur;

each E and E' is independently selected from the group consisting of Ar and $N(R_{11}R_{12})$;

each Ar is independently selected from the group consisting of phenyl and a 5-7 membered heterocycle; said phenyl and heterocycle being optionally substituted with one or more substituents R_4 (e.g., 3-pyridyl, 4-(1,2,3-thiadiazol)yl or 4-morpholine); and

each R_{11} and R_{12} is independently selected from the group consisting of C_1 - C_6 alkyl and C_2 - C_6 alkenyl.

- 73 -

4. The compound according to claim 3, wherein when both $n = 0$, E and E' are not 3-pyridyl or diethylamino.

5. The compound according to claim 3 or 4, wherein:

each n is independently selected from the group consisting of 0, 1 and 2;

each A and A' is independently selected from the group consisting of a naturally occurring alpha-amino acid and an unnatural alpha-amino acid, wherein the amino group of each A or A' is bonded to G or G' or to the carboxy group of the adjacent residue A or A', whichever is appropriate, and the carboxy group of A or A' is bonded to the amino group of the adjacent residue A or A' or to the oxygen of the structure, whichever is appropriate;

each G and G' is independently covalently attached to the amino group of the adjacent residue A or A' or to the oxygen of the structure (if the adjacent $n = 0$), and is selected from the group consisting of C_1 - C_6 alkyl; R_3CO -; $R_5(R_6R_7C)_mCO$ -; $R_5(R_6R_7C)_mW$ -; and phthaloyl, which may be optionally substituted with one or more substituents R_4 ; wherein $m = 1-3$;

each W is independently selected from the group consisting of $-OCO$ - and $-SO_2$ -, with the proviso that W is not $-SO_2$ - when W is attached to the oxygen of the structure;

each R_3 is independently selected from the group consisting of C_1 - C_6 alkyl; phenyl; and naphthyl; said C_1 - C_6 alkyl being optionally substituted with one or more substituents selected from the group consisting of hydroxyl, chlorine, and fluorine; and said phenyl

- 74 -

and naphthyl being optionally substituted with one or more substituents R_4 ;

each R_4 is independently selected from the group consisting of C_1 - C_4 alkyl; halogen; hydroxyl; nitro; and C_1 - C_3 alkoxy;

each R_5 , R_6 and R_7 is optionally and independently selected from the group consisting of hydrogen; chlorine; fluorine; a 5-7 membered heterocycle; phenyl; naphthyl; and C_1 - C_3 alkyl, wherein said C_1 - C_3 alkyl may be optionally substituted with one or more substituents selected from the group consisting of chlorine, fluorine, and hydroxyl; and wherein said phenyl and naphthyl may be optionally substituted with one or more substituents R_4 ; or R_5 , R_6 and R_7 of a particular G or G' taken individually, or together in any combination, may be optionally joined to form a monocyclic, bicyclic, or tricyclic ring system, each ring of which is C_3 - C_6 cycloalkyl; with the proviso that R_5 , R_6 , and R_7 may not be chlorine, fluorine, or hydroxyl if it is present on the carbon adjacent to W;

each B and B' is independently selected from the group consisting of oxygen and sulfur;

each D and D' is independently selected from the group consisting of H_2 ; oxygen and sulfur;

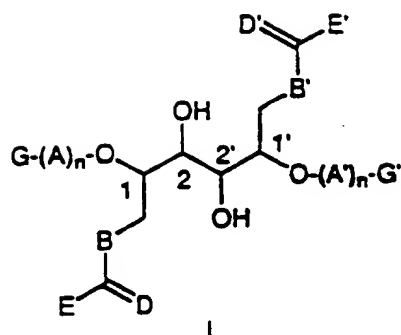
each E and E' is independently selected from the group consisting of Ar and $N(R_{11}R_{12})$;

each Ar is selected from the group consisting of phenyl and a 5-7 membered heterocycle; wherein said phenyl and 5-7 membered heterocycle may be optionally substituted with one or more substituents R_4 ; and

each R_{11} and R_{12} is independently C_1 - C_6 alkyl.

6. A compound of formula I:

- 75 -



wherein:

$n = 1$;

each A and A' is independently a naturally occurring alpha-amino acid, wherein the amino group of each A or A' is bonded to G or G' or to the carboxy group of the adjacent residue A or A', whichever is appropriate, and the carboxy group of A or A' is bonded to the amino group of the adjacent residue A or A' or to the oxygen of the structure, whichever is appropriate;

each G and G' is independently covalently attached to the amino group of the adjacent residue A or A' or to the oxygen of the structure (if the adjacent $n = 0$), and is selected from the group consisting of hydrogen and $R_5(R_6R_7C)_mW-$, wherein W is $-OCO-$ and $m = 1$;

each R_5 , R_6 , and R_7 is independently selected from the group consisting of hydrogen; chlorine; fluorine; C_1-C_3 alkyl; a 5-7 membered heterocycle; phenyl; and naphthyl; said C_1-C_3 alkyl being optionally substituted with one or more substituents selected from the group consisting of chlorine, fluorine, and hydroxyl; and wherein said phenyl and naphthyl may be optionally substituted with one or more substituents R_4 ; or each R_5 , R_6 , and R_7 of a particular G or G' taken individually, or together in any combination, may be optionally joined to form a monocyclic, bicyclic, or tricyclic ring system, each ring of which is C_3-C_6 .

cycloalkyl; with the proviso that R₅, R₆, or R₇ may not be chlorine, fluorine, or hydroxyl if it is present on the carbon adjacent to W;

each R₄ is independently selected from the group consisting of C₁-C₄ alkyl; halogen; hydroxyl; nitro; and C₁-C₃ alkoxy;

each B and B' is independently selected from the group consisting of oxygen and sulfur;

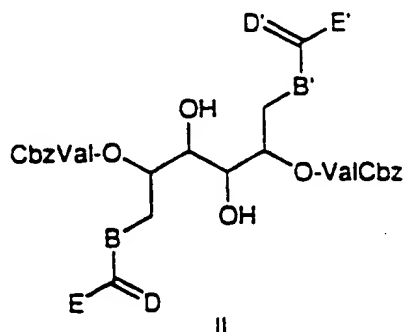
each D and D' is independently selected from the group consisting of H₂, oxygen and sulfur;

each E and E' is independently selected from the group consisting of A_r and $N(R_{11}, R_{12})$;

~~each Ar is independently selected from the~~
group consisting of phenyl and a 5-7 membered
heterocycle; wherein said phenyl and 5-7 membered
heterocycle may be optionally substituted with one or
more substituents R₄; and

each R_{11} and R_{12} is independently C_1 - C_6 alkyl.

7. The compound according to claim 6, said compound having the structure of formula II:



wherein:

each B and B' is independently selected from the group consisting of oxygen and sulfur;

- 77 -

each D and D' is independently selected from the group consisting of H₂; oxygen; and sulfur;

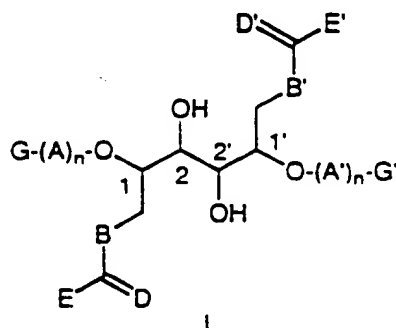
each E and E' is independently selected from the group consisting of Ar and NEt₂; and

each Ar is independently selected from the group consisting of phenyl; 3-hydroxyphenyl; 3-pyridyl; 4-(1,2,3-thiadiazol)-yl; and 4-morpholinyl.

8. The compound according to claim 1, wherein:

B, D, and E taken together are selected from the group consisting of benzoate; 3-hydroxybenzoate; nicotinoate; 4-(1,2,3-thiadiazate); benzyloxy; thiobenzoate; 4-morpholinecarbodithioate; and N,N-diethylcarbodithioate.

9. A compound of formula I,

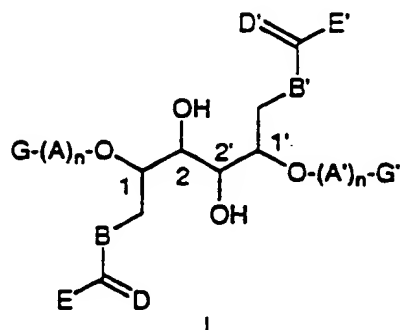


wherein:

B, D, and E taken together are selected from the group consisting of benzoate; 3-hydroxybenzoate; 4-(1,2,3-thiadiazate); benzyloxy; thiobenzoate; and 4-morpholinecarbodithioate and wherein n, A, A', G, G', B, B', D, D', E, and E' are as defined in claim 1, except that when both n = 0, E and E' are not 3-pyridyl or diethylamino.

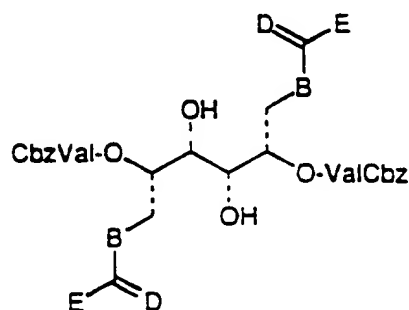
- 78 -

10. A compound of formula I:



wherein A and G taken together form carbobenzyloxyvaline and wherein n, A, A', G, G', B, B', D, D', E and E' are as defined in claim 1, except that when both n = 0, E and E' are not 3-pyridyl or diethylamino.

11. A compound having the following structure:



wherein:

B is selected from the group consisting of oxygen and sulfur;

D is selected from the group consisting of H₂; oxygen; and sulfur;

E is selected from the group consisting of phenyl; 3-hydroxyphenyl; 3-pyridyl; and 4-(1,2,3-thiadiazol)-yl; 4-morpholinyl; and diethylamino and wherein n, A, A', G, G', B, B', D, D', and E, E' are as

- 79 -

defined in claim 1, except that when both $n = 0$, E and E' are not 3-pyridyl or diethylamino.

12. A compound selected from the group consisting of:

1,6-Di-O-benzoyl-2,5-di-O-(N-carbobenzyloxyvalyl)-L-mannitol (compound 1);

1,6-Di-O-(3-hydroxy)benzoyl-2,5-di-O-(N-carbobenzyloxyvalyl)-L-mannitol (compound 2);

1,6-Di-O-nicotinoyl-2,5-di-O-(N-carbobenzyloxyvalyl)-L-mannitol (compound 3);

1,6-Di-O-[4-(1,2,3-thiadiazol)-yl]-2,5-di-O-(N-carbobenzyloxyvalyl)-L-mannitol; (compound 4);

1,6-Di-O-benzyl-2,5-di-O-(N-carbobenzyloxyvalyl)-L-mannitol (compound 5);

1,6-Di-S-benzoyl-2,5-di-O-(N-carbobenzyloxyvalyl)-L-mannitol (compound 6);

1,6-Di-S-(4-morpholinothiocarbonyl)-2,5-di-O-(N-carbobenzyloxyvalyl)-L-mannitol (compound 7); and

1,6-Di-S-(N,N-diethylthiocarbonyl)-2,5-di-O-(N-carbobenzyloxyvalyl)-L-mannitol (compound 8).

13. The use of a compound according to any one of claims 1-8 for inhibiting enzymatic activity of an aspartyl protease.

14. The use of a compound according to any one of claims 9-12 for inhibiting enzymatic activity of an aspartyl protease.

15. The use of a compound of formula I for inhibiting enzymatic activity of an aspartyl protease, wherein all of the substituents of said formula I are as defined in claim 1, except that when both $n = 0$, E and E' are not 3-pyridyl or diethylamino.

- 80 -

16. The use of a compound according to any one of claims 1-8 for inhibiting the ability of an aspartyl protease to catalyze the hydrolysis of peptide bonds.

17. The use of a compound according to any one of claims 9-12, for inhibiting the ability of an aspartyl protease to catalyze the hydrolysis of peptide bonds.

18. The use of a compound of formula I for inhibiting the ability of an aspartyl protease to catalyze the hydrolysis of peptide bonds, wherein all of the substituents of said formula I are as defined in claim 1, except that when both $n = 0$, E and E' are not 3-pyridyl or diethylamino.

19. The use according to claim 13 or 16, wherein said aspartyl protease is HIV protease.

20. The use according to claim 14 or 17, wherein said aspartyl protease is HIV protease.

21. The use according to claim 15 or 18, wherein said aspartyl protease is HIV protease.

22. The use of a compound according to any one of the claims 1-8, for inhibiting reproduction of a virus that requires an aspartyl protease for an obligatory life cycle event.

23. The use of a compound according to any one of the claims 9-12, for inhibiting reproduction of

- 81 -

a virus that requires an aspartyl protease for an obligatory life cycle event.

24. The use of a compound of formula I for inhibiting reproduction of a virus that requires an aspartyl protease for an obligatory life cycle event, wherein all of the substituents of said formula I are as defined in claim 1, except that when both $n = 0$, E and E' are not 3-pyridyl or diethylamino.

25. The use of a compound according to any one of claims 1-8, as a therapeutic agent against infection by a virus that requires an aspartyl protease for an obligatory life cycle event.

26. The use of a compound according to any one of claims 9-12, as a therapeutic agent against infection by a virus that requires an aspartyl protease for an obligatory life cycle event.

27. The use of a compound of formula I as a therapeutic agent against infection by a virus that requires an aspartyl protease for an obligatory life cycle event, wherein all of the substituents of said formula I are as defined in claim 1, except that when both $n = 0$, E and E' are not 3-pyridyl or diethylamino.

28. The use of a compound according to any one of claims 1-8, as a prophylactic agent against infection by a virus that requires an aspartyl protease for an obligatory life cycle event.

29. The use of a compound according to any one of claims 9-12, as a prophylactic agent against

- 82 -

infection by a virus that requires an aspartyl protease for an obligatory life cycle event.

30. The use of a compound of formula I as a prophylactic agent against infection by a virus that requires an aspartyl protease for an obligatory life cycle event, wherein all of the substituents of said formula I are as defined in claim 1, except that when both $n = 0$, E and E' are not 3-pyridyl or diethylamino.

31. The use of a compound according to any one of claims 1-8, for interrupting the life cycle of a virus that requires an aspartyl protease for an obligatory life cycle event.

32. The use of a compound according to any one of claims 9-12, for interrupting the life cycle of a virus that requires an aspartyl protease for an obligatory life cycle event.

33. The use of a compound of formula I for inhibiting the life cycle of a virus that requires an aspartyl protease for an obligatory life cycle event, wherein all of the substituents of said formula I are as defined in claim 1, except that when both $n = 0$, E and E' are not 3-pyridyl or diethylamino.

34. The use according to any one of claims 22, 25, 28 or 31, wherein said virus is HIV-1, HIV-2, or HTLV-1.

35. The use according to any one of claims 23, 26, 29 or 32, wherein said virus is HIV-1, HIV-2, or HTLV.

- 83 -

36. The use according to any one of claims 24, 27, 30 or 33, wherein said virus is HIV-1, HIV-2, or HTLV.

37. The use of a compound according to any one of claims 1-8, for blocking proteolysis of an aspartyl protease.

38. The use of a compound according to any one of claims 9-12, for blocking proteolysis of an aspartyl protease.

39. The use of a compound of formula I for blocking proteolysis of an aspartyl protease, wherein all of the substituents of said formula I are as defined in claim 1, except that when both $n = 0$, E and E' are not 3-pyridyl or diethylamino.

40. The use of a compound according to any one of claims 1-8, as a substrate tethered to a stable chromatographic resin in affinity chromatography.

41. The use of a compound according to any one of claims 9-12, as a substrate tethered to a stable chromatographic resin in affinity chromatography.

42. The use of a compound of formula I as a substrate tethered to a stable chromatographic resin in affinity chromatography, wherein all of the substituents of said formula I are as defined in claim 1, except that when both $n = 0$, E and E' are not 3-pyridyl or diethylamino.

43. A pharmaceutical composition effective against viral infection comprising a pharmaceutically

- 84 -

effective amount of a compound according to any one of claims 1-8 and a pharmaceutically acceptable carrier, adjuvant or vehicle.

44. A pharmaceutical composition effective against viral infection comprising a pharmaceutically effective amount of a compound according to any one of claims 9-12 and a pharmaceutically acceptable carrier, adjuvant or vehicle.

45. A pharmaceutical composition effective against viral infection comprising a pharmaceutically effective amount of a compound of formula I, wherein all the substituents of said formula I are as defined in claim 1, except that when both $n = 0$, E and E' are not 3-pyridyl or diethylamino, and a pharmaceutically acceptable carrier, adjuvant or vehicle.

46. The pharmaceutical composition according to claim 43, further comprising an additional anti-viral agent.

47. The pharmaceutical composition according to claim 44, further comprising an additional anti-viral agent.

48. The pharmaceutical composition according to claim 45, further comprising an additional anti-viral agent.

49. The pharmaceutical composition according to any one of claims 43-45, further comprising an immunomodulator.

- 85 -

50. A method for preventing HIV infection in a mammal comprising the step of administering to said mammal a pharmaceutically effective amount of a pharmaceutical composition according to claim 43, 44, or 45.

51. A method for treating HIV infection in a mammal comprising the step of administering to said mammal a pharmaceutically effective amount of a pharmaceutical composition according to claim 43, 44, or 45.

52. The method according to claim 50 or 51, wherein said pharmaceutically effective amount of said pharmaceutical composition contains between about 0.01 mg/kg body weight/per day and about 25 mg/kg body weight/per day of said compound.

53. The method according to claim 50, wherein said step of administering comprises oral administration or administration by injection.

54. The method according to claim 51, wherein said step of administering comprises oral administration or administration by injection.

55. A method for synthesizing an aspartyl protease inhibitor comprising the steps of:

(a) treating mannitol with a carbonyl compound in the presence of an acid to yield a ketal derivative; said carbonyl compound being selected from the group consisting of acetone, cyclopentanone, cyclohexanone, and benzaldehyde; and said acid being selected from the group consisting of sulfuric,

- 86 -

hydrochloric, acetic, trifluoroacetic, and mixtures thereof;

(b) esterifying said ketal derivative at each primary hydroxyl site thereof with a sulfonyl chloride to yield a bis sulfonate ester; said sulfonyl chloride being selected from the group consisting of p-toluenesulfonyl chloride, 4-bromobenzenesulfonyl chloride, methanesulfonyl chloride, and trifluoromethanesulfonyl chloride;

(c) reacting said bis sulfonate ester with a base to yield a bis epoxide; said base being selected from the group consisting of metal hydroxides, metal carbonates, and metal bicarbonates; and said bis epoxide having two least hindered sites;

(d) opening said bis epoxide at each least hindered site thereof with a nucleophile to yield a diol; said nucleophile being selected from the group consisting of an aryl alcohol, and arylthioic acid, and a heterocyclic dithioic acid;

(e) esterifying said diol with an alpha-amino acid optionally bearing an N-protecting group to yield a diester; said N-protecting group being selected from the group consisting of benzyloxycarbonyl and t-butoxycarbonyl; and

(f) treating said diester with a mixture of water and said acid of step (a) to yield said aspartyl protease inhibitor.

56. A method for synthesizing an aspartyl protease inhibitor, comprising the steps of:

(a) treating mannitol with a carbonyl compound in the presence of an acid to yield a ketal derivative; said carbonyl compound being selected from the group consisting of acetone, cyclopentanone, cyclohexanone, and benzaldehyde; and said acid being

- 87 -

selected from the group consisting of sulfuric, hydrochloric, acetic, trifluoroacetic, and mixtures thereof;

(b) esterifying said ketal derivative at each primary hydroxyl site thereof with an esterifying agent to yield a diol; said esterifying agent being selected from the group consisting of a carboxylic acid, a carboxylic acid halide, and a carboxylic acid anhydride;

(c) esterifying said diol with an alpha-amino acid optionally bearing an N-protecting group to yield a diester; said N-protecting group being selected from the group consisting of benzyloxycarbonyl and t-butoxycarbonyl; and

(d) treating said diester with a mixture of water and said acid of step (a) to yield said aspartyl protease inhibitor.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 93/12059

A. CLASSIFICATION OF SUBJECT MATTER

IPC 5 C07C271/22 A61K31/27 C07D213/80 C07D285/16 C07C327/28
 C07D295/21 C07C333/20 A61K31/44 A61K31/41 A61K31/265

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 5 C07C C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHEMICAL ABSTRACTS, vol. 101, no. 1, 2 July 1984, Columbus, Ohio, US; abstract no. 6942j, H. EIBL 'D-Mannitol derivatives as raw products for the synthesis of phospholipids' page 592 ; see abstract and RN 90126-25-3 & DE,A,32 39 858 (MAX PLANK GESELLSCHAFT) --- -/--	1-5

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
 E earlier document but published on or after the international filing date
 L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
 O document referring to an oral disclosure, use, exhibition or other means
 P document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
 Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
 & document member of the same patent family

Date of the actual completion of the international search

28 February 1994

Date of mailing of the international search report

- 9. 03. 94

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
 NL - 2280 HV Rijswijk
 Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl,
 Fax (+ 31-70) 340-3016

Authorized officer

Seufert, G

INTERNATIONAL SEARCH REPORT

Int. Application No

PCT/US 93/12059

C/(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHEMICAL ABSTRACTS, vol. 82, no. 5, 3 February 1975, Columbus, Ohio, US; abstract no. 30908n, A. I. GUREVICH ET AL. 'Synthesis of (2S,5S)-2,5-dimethoxyadipic acid' page 466 ; see abstract and RN 54322-04-2 & AKAD. NAUK SSSR, SER. KHIM. no. 9 , 1974 pages 2151 - 2153 ---	1-5,8,9
X	CHEMICAL ABSTRACTS, vol. 77, no. 1, 3 July 1972, Columbus, Ohio, US; abstract no. 5720j, C. E. BALLOU 'L-glycero-Tetralose (L-erythrulose) 1-phosphate' page 508 ; see abstract and RN 36905-07-4 & METHODS CARBOHYD. CHEM. vol. 6 , 1972 page 393-398 ---	1-5,8,9
X	CHEMICAL ABSTRACTS, vol. 103, no. 7, 19 August 1985, Columbus, Ohio, US; abstract no. 54378t, L. V. BAKINOVSKII ET AL. '1,2-O-Cyanoalkylidene derivatives of furanose as 1,2-trans-glycosylating agents' page 609 ; see abstract and RN 20834-11-1 & CARBOHYDR. RES. vol. 138, no. 1 , 1985 pages 41 - 54 ---	1-5,8,9
X	JOURNAL OF THE CHEMICAL SOCIETY, PERKIN TRANSACTIONS 1 no. 6 , 1979 , LETCHWORTH GB pages 1379 - 1383 C. MORPAIN ET AL. 'A possible model for a new chiral glyceride synthesis' see page 1379/1382 compounds 1a,b,c ---	1,2,8,9
A	WO,A,92 16501 (VERTEX PHARMACEUTICAL INC.) 1 October 1992 see page 2, line 1 - line 16; claims; table 1 ---	1-55
A	WO,A,92 00948 (SMITHKLINE-BEECHAM CORP.) 23 January 1992 see table of compounds, page 32; claims ---	1-55
A	JOURNAL OF MEDICINAL CHEMISTRY vol. 34, no. 8 , 1992 , WASHINGTON US pages 2305 - 2314 -----	1-55

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int. Application No

PCT/US 93/12059

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
DE-A-3239858	12-01-84	WO-A- 8400362 EP-A- 0098600 EP-A,B 0116566 JP-T- 59501785 US-A- 4804789 US-A- 4734225 DE-A- 3376976	02-02-84 18-01-84 29-08-84 25-10-84 14-02-89 29-03-88 14-07-88
WO-A-9216501	01-10-92	AU-A- 1677092	21-10-92
WO-A-9200948	23-01-92	AU-A- 8320691 EP-A- 0538396	04-02-92 28-04-93

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☒ FADED TEXT OR DRAWING
- ☐ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☐ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☐ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.

THIS PAGE BLANK (USPTO)